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Onaway	0.057	[illegible]	0.027	0.036	0.0[illegible]8
Brookston	0.056	0.060	0.031	.....	0.041
Miami	0.011	0.067	0.043	.....	0.058

THE AVAILABILITY OF NITROGENOUS FERTILIZERS TO RICE[1]

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The problem of maintaining an adequate supply of available nitrogen in soils for the nutrition of rice offers many difficulties because of the anaerobic condition caused by the irrigation of the crop. According to the generally accepted conception of changes taking place in the available nitrogen, excluding the assimilation by crops under anaerobic conditions, the nitrate nitrogen may be reduced to ammonia or free nitrogen. Depending upon the organisms and environment, the available nitrogen may be used directly as food by bacteria, and the ammonia from ammonium compounds may be adsorbed by the base exchange complexes in the soil. A material decrease in the available nitrogen by any one or more of the above processes might cause a decrease in the yield of rice.

With the present trend of agriculture being to increase yields per acre, the proper fertilization of crops is becoming a leading problem. Unfortunately the fertilization of rice has not proved profitable in many instances. Particularly is this true in Arkansas where the increased yield, if any, has often failed to pay for the cost of the fertilizer. The abnormal condition produced when the rice fields are irrigated suggests that the problem of maintaining available nitrogen may be one of the first problems affecting crop yields.

Some studies have already been made concerning the availability of some forms of nitrogen for rice but the results are contradictory and do not include some of the new nitrogenous compounds produced during the past few years for commercial fertilizers. Further study under controlled conditions was started to determine the reasons for the discrepancies in the results reported by other workers and to secure additional information on the question of nitrogen fertilization of rice.

The objects of the experiment were: first, to determine the availability of various nitrogenous compounds to rice; second, to study the nitrogen changes taking place under irrigated conditions.

The experiments made by a number of investigators on the availability of nitrogen to rice might be grouped, according to the conclusions drawn, into the three following groups; those showing nitrates and ammonium sulfate

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equally good for the production of rice, those concluding that ammonium compounds gave the best results, and those showing that organic compounds gave the best results.

Among the first experiments having much significance from the standpoint of rice fertilization were those of Kellner (16) in 1882. The conclusion drawn from his experiments was that nitrates alone gave higher yields than ammonium sulfate, although he goes on to state that a mixture of the two compounds gave the best results. Van Rossem (21) from field experiments reports that sodium nitrate plus superphosphate gave larger yields than did ammonium sulfate and superphosphate. Hartenbower (12) at the Guam experiment station found that nitrate of soda gave the largest yield of any single fertilizer treatment and the yield was only exceeded by the complete fertilizer treatment. Similar results have been reported more recently by Briggs (3) working at the same station. De Jong and Van Rossem (7) from a comparison of different sources of nitrogen concluded that calcium nitrate was nearly equal to ammonium sulfate for rice nutrition and that late applications of sodium nitrate gave good results. Willis and Carrero (24) state that if other factors are controlled, rice will give as good results with sodium nitrate as with ammonium sulfate. They do claim, however, that ammonium sulfate is the safest to use because there are not so many factors involved that may affect its availability as there are with sodium nitrate. Rice grown in culture solutions under controlled conditions gave good growth when nitrogen was supplied as sodium nitrate in experiments conducted by Villegas (22). In further studies using sterile cultures he states that rice flourished when potassium nitrate, ammonium sulfate, asparagin, glycocoll, and foramide were used as sources of nitrogen.

At the time the aforementioned experiments were being published, other reports claiming that ammonium sulfate was the best fertilizer for rice, were appearing in the literature. Nagaoka (18) and Daikuhara and Imaseki (6) reported from pot experiments that ammonium sulfate was the more effective fertilizer. Daikuhara and Imaseki state, however, that when rice was grown as a dry land crop, sodium nitrate and ammonium sulfate were about of equal value. Kelley (14) from the results of several years pot and field experiments concluded that ammonium sulfate was far superior to sodium nitrate.

Harrison (10) from field experiments states "Paddy soils need bulky organic manures or ones yielding ammonia under anaerobic conditions." The laterite soils in the rice district of India give good results only with cow manure according to Clouston (5). In southwestern Louisiana (4) the best results were obtained when soybeans were used in a rotation which did not include the use of fertilizers. More recently Janssen and Metzger (13) have shown that green manures may be valuable sources of nitrogen for the fertilization of rice.

With such a mass of conflicting data it seems reasonable to believe that there are certain factors other than the form of nitrogen, due to the nitrogenous compounds used, which control the growth of the plants. A recent report (2)

indicates that pH changes due to the form of nitrogen used may affect the availability of certain forms of nitrogen more than others.

PLAN OF EXPERIMENT

Experiments on the availability to rice of various nitrogenous compounds, were made in sand cultures, fertilized, with insufficient nitrogen for normal growth. Estimations made from results by Kelley (14) indicated that 0.3 gm. would be insufficient for complete normal growth and therefore the plant would utilize all of the available nitrogen. Equivalent amounts of nitrogen, 0.3 gm., were mixed in jars containing 12 kgm. of sand. The nitrogen was added as ammonium sulfate, commercial ammonium phosphate, Leunasalpeter, calcium cyanamid, sodium nitrate, calcium nitrate, urea, cotton seed meal, blood meal, and mixtures having their nitrogen one-half as cotton seed meal and one-half as sodium nitrate; and one-half as cotton seed meal and one-half as ammonium sulfate. Samples of the compounds were saved for analysis in order to calculate the exact amount of nitrogen added to each jar. Unfortunately the ammonium phosphate did not contain the amount thought to be present and the amount added did not carry as much nitrogen as the other treatments. The same amounts of ammonium phosphate were added in all experiments to keep the results comparable. All treatments were made in duplicate. After the rice was planted, a nitrogen-free nutrient solution was added. When the plants were one inch high they were thinned to 12 plants per jar and when they were six inches high they were irrigated with distilled water so that water stood two inches deep over the soil. The reaction was maintained as near pH 6.5 as possible by semi-weekly additions of dilute solutions of hydrochloric acid or sodium hydroxide, as necessary, until during a period of two weeks, constancy of the pH made further additions unnecessary.

During the whole time in which the rice was growing the surface of the sand was kept under water by additions of distilled water as often as was necessary. The rice was harvested shortly after blossoming to prevent any possible loss of nitrogen. Kelley and Thompson (15) and Sen (20) have shown that the intake of nitrogen by rice after the flowering stage is very small. The roots from each jar were washed free from sand. All samples, tops, and roots were first air-dried and then oven-dried and weighed separately. The tops and roots were ground together and thoroughly mixed for determination of total nitrogen.

GROWTH STUDIES

Chemical studies on availability of nitrogenous compounds may give some information in regard to their value as a fertilizer, but growth of plants followed by chemical analyses should give a better estimation of the availability. Two consecutive experiments were made according to the method described. In both experiments, the plants became chlorotic shortly after being flooded and remained so until almost the time of flowering, when they became green again and remained that color until changing due to maturation of the plants. That

this chlorotic condition may have been caused by a deficiency of available nitrogen and not by a lack of soluble iron as suggested by Gile and Carrero (6) will be shown later. In fact, several applications of soluble ferric phosphate and ferric citrate failed to overcome the chlorotic condition.

The average yields of oven-dried material from two jars with the different treatments are given in table 1 for both experiments.

The results on the whole agree very well when it is considered that the weight of dry matter produced is not a good criterion of availability when considered alone. The dry matter produced when urea and ammonium phosphate were used was the same in both experiments and agrees very well with that previously reported (2) in another experiment. Similarly the dry weights from the

TABLE 1

Yields of rice grown in quartz sand with the nitrogen fertilizer indicated

SOURCE OF NITROGEN	FIRST EXPERIMENT			SECOND EXPERIMENT		
	Tops	Roots	Average	Tops	Roots	Average
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
None	1.8	1.5	3.3	1.2	0.7	1.9
$NaNO_3$	20.1	15.9	36.0	13.3	12.3	25.6
$Ca(NO_3)_2$	....	....	....*	10.8	10.6	21.4
$(NH_4)_2SO_4$	15.6	9.7	25.3	16.7	16.9	33.6
$(NH_4)_3PO_4$	8.2	6.5	14.7	7.8	5.2	13.0
Urea	17.0	14.7	31.7	12.6	12.3	24.9
Calcium cyanamid	12.5	8.9	21.4	8.8	7.9	16.7
Cotton seed meal	9.2	9.9	19.1	12.8	11.3	24.1
Blood meal	9.9	9.7	19.6	13.3	12.4	25.7
Leunasalpeter	16.5	13.6	30.1	16.6	14.2	30.8
Cotton seed meal and $NaNO_3$	....	....	....	11.5	11.8	23.3
Cotton seed meal and $(NH_4)_2SO_4$	....	....	....	18.9	15.9	34.8

* Plants died.

organic treatments agree very well in the two experiments and are very close to the amounts previously reported under reaction studies. The other results vary somewhat, showing sodium nitrate to be superior to ammonium sulfate in the first experiment and inferior in the second experiment. However, the differences in neither case are large enough to indicate that one is greatly superior to the other. The probable explanation is that the two are almost equally efficient and physiological disturbances caused the differences in growth. It is of interest to note that the average yields of the two experiments from Leunasalpeter where the nitrogen is supplied as a mixture of nitrate and ammoniacal nitrogen are as good as those from any other form.

From the standpoint of dry matter produced, calcium nitrate, calcium cyanamid, blood meal, cotton seed meal, and the mixture of cotton seed meal and sodium nitrate gave yields low enough to indicate that the nitrogen contained

was less available than the other forms. In saying this it must be remembered that only half as much nitrogen was added in the ammonium phosphate treatment, and the yields would have to be doubled to be considered, or else left entirely out of consideration. When the amount of dry matter produced with

TABLE 2

Assimilation of nitrogen by rice

SOURCE OF NITROGEN	N ADDED	FIRST EXPERIMENT			SECOND EXPERIMENT		
		N in plant	N assimilated by plants	Availability	N in plant	N assimilated by plants	Availability
	mgm.	*per cent*	*mgm.*	*per cent*	*per cent*	*mgm.*	*per cent*
$NaNO_3$	295.0	0.605	187.7	63.6	0.635	162.3	55.0
$CaNO_3$	300.0	.....	.....	.....	0.510	109.0	36.4
$(NH_4)_2SO_4$	302.8	0.880	218.0	71.7	0.550	185.0	61.2
$(NH_4)_3PO_4$	152.6	0.660	97.0	63.8	0.575	74.6	48.8
Urea	264.0	0.525	166.5	63.1	0.605	151.5	57.2
Calcium cyanamid	300.0	0.600	128.4	42.8	0.800	143.8	48.0
Cotton seed meal	305.5	0.615	119.7	39.1	0.515	123.9	40.6
Blood meal	246.8	0.750	146.6	59.0	0.540	138.5	56.2
Leunasalpeter	296.5	0.660	198.0	67.0	0.585	180.3	60.8
Cotton seed meal plus $NaNO_3$	300.0	.....	.....	....	0.515	121.4	40.5
Cotton seed meal plus $(NH_4)_2SO_4$	304.2	.....	.....	....	0.510	177.5	58.4

TABLE 3

Relative availability of nitrogenous fertilizers for rice, ammonium sulfate considered as 100

SOURCE OF NITROGEN	RELATIVE AVAILABILITY		
	First experiment	Second experiment	Average
$NaNO_3$	89	90	89.5
$CaNO_3$	...	59	59.0
$(NH_4)_2SO_4$	100	100	100.0
$(NH_4)_3PO_4$	89	80	84.5
Urea	88	94	92.0
Calcium cyanamid	60	79	69.5
Cotton seed meal	55	66	61.5
Blood meal	82	92	87.0
Leunasalpeter	93	99	96.0
Cotton seed meal plus $NaNO_3$	...	66	66.0
Cotton seed meal plus $(NH_4)_2SO_4$	...	96	96.0

half the amount of nitrogen is compared with the other yields, ammonium phosphate appears to be a suitable source of nitrogen for the fertilization of rice. Sodium nitrate, Leunasalpeter, ammonium sulfate, urea, the mixture of organic and ammoniacal nitrogen, and ammonium phosphate all seem to be almost equally good from the weights of dry matter produced.

As yields do not tell the complete story of the availability of nitrogenous compounds the plants were analyzed for total nitrogen and the amounts of nitrogen assimilated from the various sources calculated. The results are given in table 2.

The results are a little different than those from the total yields of dry matter. Rice growing on the ammonium sulfate treatment assimilated the most nitrogen. However, the amounts taken up from the sodium nitrate, ammonium phosphate, Leunasalpeter, and cotton seed meal mixture are almost as great as that from the ammonium sulfate. On the basis of amounts assimilated they could all be considered as good fertilizers for rice.

The results of the analyses of the plants from the cotton seed meal and blood meal treatments show the blood meal to be more available although the total dry weights show them to be of about equal value. The poor results obtained in both experiments with calcium nitrate are rather puzzling. Five different trials have been made in various experiments to get rice to grow when the nitrogen was supplied as calcium nitrate but in all cases the growth has been rather poor. In one experiment the failure to grow was due to a rapid decrease of the pH to alkalinity. It may be possible in the experiments with calcium nitrate when the reaction was fairly well controlled that the chlorosis observed may have resulted from an antagonistic action from the soluble calcium and may have decreased the yield in a manner similar to that reported by Willis and Carrero (25) for calcareous soils.

The relative availability of the different compounds, ammonium sulfate being used as 100, are given in table 3.

The relative availabilities for the separate experiments with the exception of calcium cyanamid agree very well. The averages for the two experiments show that ammonium sulfate, Leunasalpeter, urea, sodium nitrate, blood meal, and a mixture of cotton seed meal and ammonium sulfate should produce good yields of rice if no other factors affect the ability of the plant to utilize the nitrogen or affect the availability of the nitrogen in the soil.

NITROGEN CHANGES

The changes taking place in nitrogenous compounds under anaerobic conditions may depend to some extent upon the form which is present when the anaerobic condition develops. It is generally understood however, that the loss of some total nitrogen as well as a great decrease in available nitrogen may take place when anaerobic conditions develop. Greaves (9) states that the loss of nitrogen by denitrification may occur from the breaking down of complex proteins into simpler products from the reduction of nitrates to ammonia or free nitrogen, and from the transformation of nitrates or ammonia to more. complex proteins.

In order to study the effect of flooding upon nitrogenous fertilizers, 300 mgm. of nitrogen was added to 12 kgm. of quartz sand to which was applied a nitrogen-free nutrient solution and 100 cc. of a water extract made from a field soil

which had been cropped to rice. The moisture content was maintained as near 15 per cent as possible for five weeks by the addition of distilled water. At this time the cultures were flooded with distilled water so that water stood one inch deep on the surface of the sand. The water was maintained near this level until the end of the experiment by the daily addition of distilled water. The reaction of the cultures was maintained as near pH 6.5 as possible by the semi-weekly addition of dilute hydrochloric acid or sodium hydroxide as necessary until a period of two weeks, constancy of the pH made further additions unnecessary.

After irrigation, samples were taken every week to determine the total amounts of water-soluble nitrogen present as ammonia, nitrites, and nitrates. In future discussion the phrase "water-soluble nitrogen" will imply those three forms. The samples were taken in the following manner: the cultures were stirred thoroughly and left standing for 24 hours to let all solubility reactions come as near to equilibrium as possible. Then samples of liquid, equivalent to one-fortieth of the original amount of nitrogen, were taken by weighing the jars and taking the required amount from the supernatant liquid. Although objections may be raised as to the accuracy of this method of sampling, preliminary experiments had shown that it was practically impossible to get similar aliquots of the sand and water. For this reason aliquots taken as described probably more nearly represented the amount of soluble nitrogen present than could have been obtained by trying to get the proper mixture of sand and liquid.

The samples were made to 250 cc. with distilled water and thoroughly mixed. Duplicate aliquots were taken for determination of nitrate, nitrite, and ammoniacal nitrogen. The method used for analysis for nitrate and nitrite nitrogen have been reported in a previous paper (1). The ammonia was determined by distilling 50-cc. aliquots with sodium carbonate and catching the distillate in 0.01 N HCl. The distillate was made to a volume of 200 cc. with distilled water, aliquots were taken and treated with Nessler's reagent. The amount of nitrogen present as ammonia was determined colorimetrically by comparison with standard solutions which had been treated with Nessler's reagent.

In order to determine the amounts of nitrogen lost by denitrification processes the free water was permitted to evaporate from the uncropped jars nine weeks after irrigation. The jars were weighed and the sand was thoroughly mixed in a galvanized iron tub. Samples were taken for total nitrogen analyses. The analyses were made in triplicate using the salicylic acid modification to include nitrogen in all forms. The ammonia in the distillate was determined by the use of Nessler's reagent.

For ease of study the compounds have been grouped according to the form of nitrogen they contained.

Nitrate nitrogen

The results of duplicate analyses of the changes taking place in nitrogen under anaerobic conditions are given in table 4.

A study of the results gives some very interesting information. The loss of nitrogen from nitrates regardless of whether it is being used as a source of oxygen for bacteria or whether it is being reduced to ammonia is generally regarded as taking place in several stages, during one of which nitrites are supposed to be found. The data reveal that only in one analysis of samples from the sodium nitrate treatment was any appreciable amount of nitrite nitrogen found and only very small amounts were found in the samples from the calcium nitrate treatment. The latter statement is particularly interesting when we consider the amounts of nitrogen as ammonia evidently reduced from nitrates found on several occasions. A possible explanation may be that the reduction of nitrites to ammonia may take place just as rapidly as has been shown (1) for the change from nitrites to nitrates. If such were the case only during certain periods of activity, similar to those suggested by Miyake (7)

TABLE 4

Effect of anaerabic conditions on nitrate nitrogen

WEEKS AFTER PLANTING	WEEKS AFTER IRRIGATION	N in $NaNO_3$ changed to			N in $Ca(NO_3)_2$ changed to		
		NH_3	NO_2	NO_3	NH_3	NO_2	NO_3
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
5	0	None	Trace	84.4	None	Trace	26.4
6	1	None	Trace	33.6	16.0	Trace	24.8
7	2	None	Trace	83.6	37.2	Trace	62.4
8	3	None	0.28	127.0	3.6	Trace	86.8
9	4	None	Trace	111.2	4.4	Trace	44.1
10	5	8.4	4.50	97.2	24.0	0.80	90.0
11	6	None	0.20	93.5	None	0.40	31.2
14	9	Trace	14.84	20.0	None	None	Trace

for aerobic conditions, would nitrites be present and the finding of large amounts of nitrite nitrogen in the samples would be due to the accidental sampling of the jars during a period of great activity.

It seems very improbable from the results of these experiments that the reduction of nitrate to nitrite in sufficient quantities to be toxic to plants takes place as suggested by Kelley (14) and cannot account for the failures reported (6, 14, 18) of sodium nitrate to produce as good yields as ammonium sulfate. This agrees with results presented by Janssen and Metzger (13) who found only very small quantities of nitrites present, under irrigated conditions, from applications of sodium nitrate to a rice soil.

At no time was there much over one-third of the 300 mgm. of nitrogen originally added in a soluble form. This would suggest that loss had been due either to rapid denitrification or to the assimilation of a large amount by bacteria. That the latter action is an important cause of the decrease in soluble nitrogen will be shown later.

Ammoniacal- and ammonia-producing nitrogen

Ammonia-producing compounds are those such as urea and calcium cyanamid which upon coming into contact with water hydrolyze to form ammonia. Urea decomposes according to the following formula,

$$\text{(a)} \qquad CO(NH_2)_2 + 2H_2O \rightarrow (NH_4)_2CO_3.$$

Cyanamid hydrolyzes first into urea according to these reactions,

$$\text{(b)} \qquad CaCN_2 + 2\ H_2O \rightarrow H_2CN_2 + Ca(OH)_2$$
$$\text{(c)} \qquad H_2CN_2 + H_2O \rightarrow CO(NH_2)_2$$

and then from urea into ammonia according to the formula (*a*) given for the hydrolysis of urea. The changes in the soil after hydrolysis had taken place would be the same as for those fertilizers applied directly as ammonium salts. The changes taking place from the nitrogen in these compounds under anaerobic conditions may be studied from the results given in table 5.

With the exception of two samplings of the ammonium sulfate treatment the water-soluble nitrogen was never during the experiment over one-third of that originally added. In many cases the water-soluble nitrogen was only a few per cent of that originally added. Although it might be surmised that some of the nitrogen had been lost by denitrification processes it is also probable that some was converted to an insoluble form, by assimilation by bacteria and by adsorption by the sand as suggested by Wolkoff (26).

The results of the analyses from the urea treatment are rather interesting. Starting with a trace of nitrate nitrogen at the time of irrigation there was an increase in the nitrates present for three weeks and then a decrease until only a trace remained. There was a large increase in all three forms of nitrogen at the third sampling of the urea treatment, whereas in the samples taken a week later there was an increase in nitrate nitrogen only and a decrease in the other two forms. Whether the nitrites present were the intermediate stage of nitrification made possible by oxygen stirred into the cultures in preparation for the sampling can only be a matter of speculation, for it can not be proved from the data presented and, as far as is known, there have been no data pertaining to similar conditions presented in the literature. The data from the seventh sampling of the calcium cyanamid treatments also indicate that some oxidation of ammonia is taking place, because there is a decided increase in nitrite nitrogen accompanied by a large decrease in ammoniacal nitrogen.

In only one sample, the third sampling from the urea treatment, was there any large amount of nitrites present. This seems rather remarkable when such quantities of nitrates disappeared in a week or two from different treatments. Such a rapid loss of nitrate nitrogen without the appearance of nitrite nitrogen tends to substantiate the contention previously made that the nitrate is reduced so rapidly that the finding of large quantities present at any one time may be due to the accidental sampling during a cycle of activity of nitrate-reducing bacteria.

TABLE 5

Effect of anaerobic conditions on ammoniacal-nitrogen- and ammonia-producing compounds

WEEKS AFTER PLANTING	WEEKS AFTER IRRIGATION	N IN $(NH_4)_2SO_4$ CHANGED TO			N IN $(NH_4)_3PO_4$ CHANGED TO			N IN UREA CHANGED TO			N IN CALCIUM CYANAMID CHANGED TO		
		NH_3	NO_2	NO_3	NH_3	NO_2	NO_3	NH_3	NO_2	NO_3	NH_3	NO_2	NO_3
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
5	0	68.0	Trace	Trace	45.6	Trace	Trace	62.4	1.92	Trace	22.0	Trace	Trace
6	1	44.4	Trace	Trace	58.8	Trace	Trace	14.4	0.78	9.0	34.0	Trace	Trace
7	2	177.2	0.24	Trace	49.2	0.16	Trace	67.0	40.0	31.2	1.6	Trace	3.3
8	3	202.0	0.12	Trace	51.6	0.68	Trace	42.8	Trace	41.6	Trace	Trace	Trace
9	4	92.0	None	Trace	Trace	0.43	Trace	2.4	Trace	11.8	Trace	Trace	Trace
10	5	104.0	Trace	Trace	Trace	Trace	Trace	4.0	0.64	9.0	32.0	Trace	Trace
11	6	67.2	None	Trace	Trace	Trace	Trace	Trace	0.24	Trace	Trace	7.16	Trace
14	9	Trace	None	Trace	Trace	None	Trace	Trace	None	Trace	Trace	None	Trace

Organic nitrogen

The transformations taking place in organic nitrogen under anaerobic conditions would be radically different from those described for the preceding processes because the nitrogen is in a complex form and would have to be liberated in a simpler form before it would be available to plants. It is true that because several weeks elapsed before irrigation some of the nitrogen could

TABLE 6

Effect of anaerobic conditions on organic nitrogen

WEEKS AFTER PLANTING	WEEKS AFTER IRRIGATION	N IN COTTON SEED MEAL CHANGED TO			N IN BLOOD MEAL CHANGED TO		
		NH_3	NO_2	NO_3	NH_3	NO_2	NO_3
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
5	0	67.2	4.76	Trace	28.0	2.32	Trace
6	1	4.0	0.26	Trace	8.4	0.68	Trace
7	2	49.2	0.06	8.9	49.2	0.13	Trace
8	3	39.6	0.16	28.4	54.4	0.15	Trace
9	4	8.6	0.30	Trace	7.6	Trace	Trace
10	5	14.0	3.88	Trace	4.40	0.28	Trace
11	6	Trace	0.35	Trace	Trace	0.32	Trace
14	9	Trace	None	Trace	Trace	None	Trace

TABLE 7

Effect of anaerobic conditions on mixtures of nitrate and organic and ammoniacal nitrogen

WEEKS AFTER PLANTING	WEEKS AFTER IRRIGATION	N IN LEUNASALPETER CHANGED TO			N IN COTTON SEED MEAL AND $NaNO_3$ CHANGED TO			N IN COTTON SEED MEAL AND $(NH_4)_2SO_4$ CHANGED TO		
		NH_3	NO_2	NO_3	NH_3	NO_2	NO_3	NH_3	NO_2	NO_3
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm*	*mgm.*
5	0	88.4	Trace	20.0	6.8	2.88	19.4	29.2	3.52	Trace
6	1	10.8	Trace	17.8	Trace	3.12	24.2	16.0	0.50	Trace
7	2	94.8	0.12	35.7	Trace	16.95	55.6	127.2	0.04	Trace
8	3	99.6	Trace	66.5	Trace	0.40	91.0	140.4	0.28	Trace
9	4	48.8	Trace	25.3	Trace	0.90	50.0	33.4	Trace	Trace
10	5	30.8	0.64	Trace	Trace	1.60	6.4	30.6	Trace	Trace
11	6	12.8	Trace	Trace	Trace	3.71	14.7	Trace	Trace	Trace
14	9	Trace	None	Trace	Trace	None	Trace	Trace	Trace	Trace

have been converted to more available forms than contained in the organic form. The changes taking place can be explained from the analyses in table 6.

For the major part of the experiment, nitrogen as ammonia was being liberated from the organic nitrogen. In connection with this the small concentrations of nitrite nitrogen present in nearly all samplings, because they are more consistent than in almost any other treatment, suggest that the changes of organic nitrogen may have been carried farther than the ammonia form. The

data for the samples taken the second and third week after flooding are suggestive of nitrification reactions and agree with suggestions previously made. As would be expected from the form of nitrogen used, there were never any large amounts of water-soluble nitrogen present in the cultures.

Mixed forms of nitrogen

Each form of nitrogen in a mixture of nitrogenous fertilizers might be expected to behave as though present alone. That such is the case may be seen by the results presented in table 7.

The results agree on the whole with those of the other experiments in that at no period of sampling was there much over one-third of the nitrogen present in a soluble form. The amounts of nitrite nitrogen found at the different periods of sampling were also small.

TABLE 8

Losses of nitrogen from various nitrogenous fertilizers in anaerobic sand cultures

SOURCE OF NITROGEN	N ADDED	FOUND BY ANALYSES	LOST
	mgm.	*mgm.*	*per cent*
$NaNO_3$	295.0	95.2	67.8
$CaNO_3$	302.0	165.2	45.3
$(NH_4)_2SO_4$	302.8	223.2	26.2
$(NH_4)_3PO_4$	152.6	135.2	11.4
Urea	264.0	143.8	45.5
Calcium cyanamid	300.0	120.8	59.8
Cotton seed meal	305.5	88.8	71.0
Blood meal	300.0	137.5	54.2
Leunasalpeter	296.5	123.2	58.4
Cotton seed meal and $NaNO_3$	300.2	76.0	74.7
Cotton seed meal and $(NH_4)_2SO_4$	304.1	205.6	32.4

Losses of nitrogen by dentrification

Losses of nitrogen by denitrification from rice soils might greatly decrease the yields of rice. From a theoretical consideration (23) nitrogen from nitrates would be lost in the greatest amounts. This does not preclude that possibility that losses from organic and ammoniacal forms may be just as great as from nitrate nitrogen. The results in table 8 were secured from an experiment to determine the amounts lost from the various compounds which could be used for nitrogenous fertilizers.

The most striking result is the fact that considerable amounts of nitrogen were lost from all sources except the strict ammoniates. The losses from nitrates were larger than those from the true ammoniates, such as ammonium sulfate and ammonium phosphate, but on the other hand the ammonia-producing compounds and the organic nitrogenous compounds lost as much as or in some cases more than the nitrates. Although some of the losses from the compounds

may be attributed to loss from nitrates formed by nitrification processes before irrigation, tables 4 to 7 show that in some instances practically no nitrate nitrogen was produced. Ammonium sulfate is a very good example to use. There is shown a total loss of 26.0 per cent and yet at no time was there more than a trace of nitrates present. Blood meal, calcium cyanamid, and the mixture of cotton seed meal and ammonium sulfate acted in a similar way, showing a loss of 54.0, 59.8, and 32.4 per cent respectively of the nitrogen added and yet there was never more than a trace of nitrates present during the entire experiment.

The results do not show that denitrification of nitrates with the production of nitrites, as suggested by Kelley (14), is the chief cause of the failure to produce good crops of rice. In only one case, a late sampling of sodium nitrate treatment in a series of experiments mentioned in the following, did the nitrite concentration approach that which he considers toxic. In most cases the concentration was much less than 1 p.p.m. and according to Perciabosco and Rosso (19) was dilute enough for direct assimilation by rice.

The losses occurring from the organic nitrogenous compounds were probably due to the anaerobic decomposition of the compound, a process similar to that taking place in paddy soils, as reported by Harrison and Aiyer (11). They state that the gaseous nitrogen found in paddy soils arises from the decomposition of the organic matter, green manure, and decomposing roots in the soil.

A second series of experiments were made but because of the higher temperature at which they were run and the shorter duration of the experiments they could not be compared directly with the results reported above. However, the same conclusions may be drawn from the results as from the above experiments; viz., (*a*) At no time was much over one-third of the nitrogen originally added present in a soluble form; many times only a few per cent was present in a soluble form. (*b*) Nitrites were only found in small amounts in the different treatments, including the nitrate nitrogen fertilizers. (*c*) Nitrogen was lost by denitrification processes from all the different forms of nitrogen, but the losses from the strict ammoniates such as ammonium sulfate and ammonium phosphate, were somewhat smaller than from all the other forms.

DISCUSSION OF RESULTS

Nitrogen whether in nitrate, organic, or ammonia form seems to be readily available for rice if other growth factors such as reaction, temperature, and light are maintained uniform for all treatments. There may be some differences in certain organic compounds due to differences in the rate at which they decompose and liberate nitrogen. Although no attempt will be made to discuss the results from the standpoint of explaining each conflicting experiment mentioned in the literature review, certain facts which appear to be fundamental will be discussed from the data presented. It has been suggested (14) that nitrates should not be used for rice because it would not produce good yields.

The evidence presented shows that when the reaction is maintained constant, nitrogen from sodium nitrate is assimilated almost as readily as from ammonium sulfate. There is a possibility that part of the nitrate nitrogen may be reduced to ammonia or it may be assimilated by bacteria and later liberated from their bodies in the ammonium form and utilized as such by the plants.

When no precautions are taken to prevent the media from becoming more alkaline there is a decidedly injurious effect from the use of sodium nitrate for fertilization of rice, as has been shown previously (2). The use of sodium nitrate, or in fact any physiologically basic fertilizer, on a slightly acid to neutral soil may result in no response or even a decreased yield from the fertilizer. On the other hand nitrates could probably be used on distinctly acid soils to good advantage.

The reduction of nitrates to free nitrogen did not seem to take place any faster from nitrates than from any other compound, exclusive of ammonium sulfate and ammonium phosphate, so that only under very limited conditions might the amount of nitrogen lost from nitrates by denitrification seriously affect the crop yield. The loss after four months in uncropped cultures was 67.8 per cent of the nitrogen added. As a matter of fact, if the jars had been cropped the loss would probably not have been nearly so great, for analyses of the crops showed that they assimilated 63.6 per cent of the nitrogen added as sodium nitrate. This would give a loss of 36.4 per cent by deditrification, assuming that all the nitrogen not taken up by the plant was denitrified, which was probably not the case. Part of the 36.4 per cent had probably been assimilated by bacteria and retained in their bodies.

The organic compounds, if they are readily decomposed, are well adapted for the fertilization of rice. However, there appears to be as much danger of losing nitrogen from them as from sodium nitrate. Having practically no effect on the soil reaction, they could probably be used under practically all soil conditions. If considerable time elapses from their application to their irrigation nitrification may take place and the situation would be somewhat similar to that where sodium nitrate was applied. A similar condition would be produced from the plowing under of crop residues and green manures.

Ammonium fertilizers seem best adapted for all conditions. Although they may produce a considerable increase in the pH, the rice plants seem to be able to stand rather large changes without showing much injury. The compounds could be used on most soils regardless of reaction. Also they do not seem to lose as much nitrogen by denitrification process as the other compounds. Part of this may be due to the fact that ammonia may be adsorbed by soil complexes and is not available in as large quantities for biological activities as are some of the other compounds.

Why so much more nitrogen should be lost from fertilizers such as urea and cyanamid, which produce ammonium carbonate by hydrolysis, than from the strict ammoniates can not be readily explained. Part of it may have been due to a denitrification process similar to that taking place from organic matter.

Growth with Leunasalpeter, in which the nitrogen is three-fourths ammonia-

cal and one-fourth nitrate, was within 4 per cent as effective in producing rice as was ammonium sulfate when ammonium sulfate was taken as 100. This seems to indicate that any form of nitrogen as long as it changed either to the nitrate or ammonium form would be readily assimilated by rice.

Mention has been previously made that all the rice became rather chlorotic shortly after flooding. This condition was very similar to that observed under field conditions even when nitrogenous fertilizers are applied. The results of the nitrogen transformation studies suggest that the chlorotic condition was due to an insufficient supply of available nitrogen.

SUMMARY

The availability of the various forms of nitrogen was determined by plant growth and chemical analysis of the plant tissue to determine the amount of nitrogen assimilated. Studies were made of the transformations taking place in various nitrogenous compounds under anaerobic conditions to determine whether these changes might affect the suitability of these compounds for the nutrition of rice. A brief summary of the results follows.

When proper precautions were taken the efficiency of the following nitrogenous compounds, compared to ammonium sulfate as 100 were, Leunasalpeter 96 per cent, a mixture of cotton seed meal and ammonium sulfate 96 per cent, urea 92 per cent, sodium nitrate 89.5 per cent, blood meal 87 per cent, ammonium phosphate 84.5 per cent, calcium cyanamid 69.5 per cent, a mixture of cotton seed meal and sodium nitrate 66 per cent, cotton seed meal 61.5 per cent, and calcium nitrate 59 per cent. Under proper conditions the first six seem well adapted for the production of rice. However, it is safer to recommend the ammonium compounds, such as ammonium sulfate and ammonium phosphate, because rice seems to be affected less by the changes they produce and there is the probability that less nitrogen may be lost by denitrification.

Organic forms of nitrogen may be recommended highly because they produce good results and have practically no effect on the soil reaction. There is danger, however, of loss of considerable amounts of nitrogen through denitrification. Although sodium nitrate gave good results under controlled conditions its general use should not be recommended because of the sensitiveness of rice to decreases in pH. The same thing would apply for all physiologically basic fertilizers.

The production of nitrites due to denitrification was very spasmodic and only in several cases were they found in quantities larger than several mgm. per jar. Nitrites were found in jars which had received no nitrate nitrogen. The results do not support the contention that the production of nitrites from nitrates is the cause for the failure of nitrates to produce good yields of rice.

Nitrogen was lost, presumably as elemental nitrogen, from all form of nitrogen whether it was in the ammonium, nitrate, or organic form, but smaller amounts were lost from the ammonium compounds, such as ammonium sulfate and ammonium phosphate, than from nitrates and organic nitrogen. Dentrification of nitrates was no greater than that of organic compounds such as

blood meal, cotton seed meal, and urea. The dentrification process took place so rapidly that only small quantities of nitrogen as nitrites were found from most treatments.

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SOIL TYPE AND CROP ADAPTATION

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That there is a vast difference in the response of the same crops to different soil types in Florida is common knowledge. In fact, if this is not given due consideration, trucking and fruit growing in portions of the state are more or less hazardous. With the knowledge that this condition does exist and with the realization of the value of a practical working knowledge of it in connection with successful farm operations, an attempt has been made to collect as much detailed information as possible to bring out the relationship between soil type and crop adaptation at Penny Farms.

EXPERIMENTAL

Nine of the most prominent soil types occurring at Penny Farms have been used in connection with this work. They are Norfolk fine sand flat phase, Norfolk fine sand, Leon fine sand, Leon fine sand loamy phase, Blanton fine sand, St. John's loamy fine sand, Portsmouth loamy fine sand, Portsmouth fine sand, and Portsmouth fine sandy loam. These soils vary widely in their general characteristics and crop-producing power. The surface of the Norfolk fine sand, to a depth of 5 to 6 inches, is light gray, incoherent fine sand. The subsoil, to a depth of 36 inches or more, is a yellowish gray to pale yellow, loose fine sand. It is deficient in humus and is considered to be very poor for general truck crops. The Norfolk fine sand, flat phase, consists of a gray to dark gray, incoherent fine sand, underlain at about 8 to 10 inches by a yellow, pale yellow, or grayish yellow incoherent fine sand, extending to a depth of more than 3 feet. This type is somewhat superior to the Norfolk fine sand. The Leon fine sand consists of a gray to whitish gray, loose and incoherent fine sand, which at from 15 to 34 inches is underlain by a reddish brown to dark brown (and sometimes black) compact sand or organic hardpan layer. The immediate surface for 2 to 4 inches may be a gray or dark gray fine sand, the dark coloring being organic coloring from decayed grass roots. The hardpan layer ranges from a few inches to 2 or 3 feet in thickness. Usually it is from 6 to 14 inches in thickness. Below the hardpan layer is a moist, loose, incoherent fine sand having the nature of quick sand, when saturated. The Leon fine sand, loamy phase, is a gray to dark gray fine sand, 1 to 5 inches deep, overlying a light gray to almost white, rather incoherent fine sand which at

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depths varying from 8 to 30 inches, though usually at about 15 to 22 inches, passes into a dark brown or rusty brown and sometimes black, dense hardpan layer. This rages from 3 inches to 2 feet in thickness, and is underlain by a white, fine sand which is always moist and compact, but when disturbed becomes incoherent and has the nature of quicksand. Blanton fine sand consists of 2 to 4 inches of a gray to dark gray fine sand underlain by a yellowish gray to grayish white fine sand 25 to 40 inches thick, resting upon a very light grayish yellow to light gray fine sand.

Portsmouth loamy fine sand is a very dark brown to black loamy fine sand containing a high percentage of organic matter. This dark organic material extends to 15 to 24 inches then changes to a dark gray to gray fine sand, gradually becoming lighter with depth. Portsmouth fine sandy loam is identical with the Portsmouth loamy fine sand, except that it is slightly higher in organic matter than the latter and has a sticky gray sandy clay loam in the lower subsoil at 34 to 36 inches. Portsmouth fine sand differs mainly from Portsmouth loamy fine sand in that it has a layer of white sand from 15 to 25 inches below the surface. St. John's loamy fine sand, to a depth of 5 to 10 inches, consists of a very dark gray to black loamy fine sand. Below this is a light gray to grayish white, incoherent fine sand which is underlain at depths ranging from 10 to 36 inches, but ordinarily 22 to 28 inches, by a dark brown to black, compact, organic hardpan. The layer of hardpan, which ranges from 2 to 6 inches in thickness, rests upon a light gray, incoherent fine sand.

For convenience the two Norfolk types are grouped as Norfolk and the three Portsmouth types as Portsmouth and are treated as such in the remainder to this article.

In connection with the development of the farm operations of the J. C. Penny-Gwinn Corporation at Penny Farm, Florida, it was deemed necessary that a detailed soil survey on the scale of 2 inches to the mile be made of the entire tract and that a careful study be made of the response to different soil types of all crops grown. In keeping with this plan some very complete and extensive data have been collected to show that there is a striking relationship between soil type and crop adaptation at Penny Farms.

For this study Irish potatoes, grapes, peaches, pears, pecans, plums, blueberries, tung oil, crotalaria, corn, pepper, Japanese sugar cane, peppermint, sweet potatoes, and numerous other crops are used. These crops will be discussed in the order mentioned.

All data reported here for Irish potatoes were obtained from fields where the soil survey showed the soil to be uniformly of one type throughout. All acreages in connection with the potato crop were accurately chained, and a complete set of field notes kept on each field used throughout the season. All yield data represent accurately measured yields. The data were all obtained in connection with actual field operations.

Some 400 acres or more were planted to potatoes between January 20 and February 10, 1927, on the leading soil types mentioned, for the spring crop.

Uniform fertilizer treatment and cultural practices were used on the entire acreage, thus making it possible to obtain comprehensive data bearing on the relation of soil type to potato production during the season.

The data contained in table 1 were obtained by taking the average yield of no. 1 potatoes for a few of the representative fields located on Norfolk soil as 100 per cent and similar averages for each of the other soil types and expressing these in per cent. An examination of the data clearly reveals a decided relationship between soil type and potato production with the spring crop. The heavier St. John's and Portsmouth soils proved to be far superior to the lighter, drier Norfolk, Leon fine sand loamy phase, and Blanton soils.

TABLE 1

Relation of soil type to potato yield in the spring of 1927

SOIL TYPE	YIELD ON PERCENTAGE BASIS
Norfolk	100
Leon fine sand, loamy phase	170
Blanton fine sand	171
St. John's loamy fine sand	224
Portsmouth	264

TABLE 2

Relation of soil type to potato yield in the fall of 1927

SOIL TYPE	YIELD EXPRESSED ON PERCENTAGE BASIS
Norfolk	100
Leon fine sand, loamy phase	140
Blanton fine sand	155
St. John's loamy fine sand	305
Portsmouth	321

The Norfolk soils made the poorest showing of all the types used. They proved to be very unsatisfactory for potatoes in every case. This is largely because of their lack of humus, their open, sandy character, and their inability to retain moisture during the growing season.

The Blanton fine sandy soil, which is somewhat heavier, slightly more compact, and less droughty than the Norfolk soils, proved to be a superior potato soil to the latter. This soil, however, did not rate as a first-class potato soil, as may readily be seen. It, too, is poor at retaining moisture.

The loamy phase of Leon fine sand proved to be only a medium potato-producing soil. Because of the peculiar nature of this soil, it, too, has a tendency to be droughty or very poor at retaining moisture.

The St. John's loamy fine sand, which is much heavier and more compact than either of the aforementioned soils, was superior to them in producing

potatoes. The high organic matter content, its compact nature, and its natural low position make it very efficient at holding moisture, which accounts in part for its superiority as a potato soil.

The Portsmouth soils easily proved their superiority for potato production over all the other types during this period. This is largely because of their larger humus content, more compact nature, and ability to retain moisture during drought.

Potatoes were planted on the same types of soil during the fall of 1927, but on a much smaller acreage, with the results listed in table 2.

The figures in table 2 were obtained by taking the average total acre yield of graded potatoes for all the Norfolk acreage planted as 100 per cent and similar averages for each of the other soil types and expressing these in per cent.

TABLE 3

Soil type and potato yields for the spring of 1928

SOIL TYPE	NUMBER OF ACRES PLANTED	TOTAL YIELD IN BARRELS	AVERAGE ACRE YIELD IN BARRELS	YIELD EXPRESSED ON PERCENTAGE BASIS
Norfolk fine sand, flat phase	16.10	919	57.0	100
Leon fine sand, loamy phase	11.20	566	50.7	89
Blanton fine sand	80.05	5,091	63.7	112
St. John's loamy fine sand	5.00	293	58.6	103
Portsmouth	132.20	8,534	64.5	113

TABLE 4

Average monthly rainfall in inches from 1876 to 1926

January	2.75	July	6.50
February	3.75	August	6.30
March	3.90	September	5.30
April	3.30	October	3.75
May	5.00	November	1.90
June	6.75	December	3.60

The percentage figures for the various soil types of the fall crop are not exactly the same as the corresponding figures for the spring crop, to be sure, but there is indeed a very close correlation between them. In every instance, the various soil types for the fall crop maintained the same positions in the scale of relative values as for the spring crop. This shows conclusively that there was a decided relationship between soil type and potato production during the spring and fall of 1927.

The results reported above were obtained during seasons when the rainfall was about average or normal for this section. Since moisture is often the limiting or controlling factor in potato production on these soils, it is natural to expect those soils of low water-holding capacity to suffer more severely than

those capable of retaining large quantities of moisture during normal or dry seasons. However, with the moisture factor satisfactorily solved, either by means of an adequate amount of rainfall properly distributed through the growing season or by irrigation, this relationship will be much less noticeable. On the other hand, should there be a surplus rainfall the heavy soils, having a

TABLE 5

Daily rainfall in inches for first six months of 1928

DAY	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE
1	0.08	....	....	....	....	....
2	....	....	....	....	....	0.25
3	....	....	....	....	....	....
4	....	....	....	....	....	....
5	....	....	....	....	....	....
6	....	....	....	....	....	....
7	....	....	....	....	0.95	0.06
8	....	....	....	....	0.08	....
9	0.07	....	....	....	....	....
10	....	....	....	1.40	....	1.15
11	....	....	0.52	0.91	....	....
12	....	....	....	....	....	....
13	....	0.42	....	....	0.05	....
14	....	....	....	....	....	0.08
15	....	....	....	3.60	....	2.50
16	....	0.33	0.06	....	....	1.40
17	....	....	0.70	....	....	....
18	....	0.47	0.07	....	....	....
19	....	....	....	....	....	....
20	0.04	....	....	....	....	....
21	....	....	....	....	0.19	0.20
22	0.22	....	....	0.60	0.40	0.90
23	....	0.04	....	1.25	0.84	0.22
24	....	1.50	....	....	....	0.20
25	....	0.12	0.20	....	....	0.85
26	....	....	1.20	....	....	0.25
27	0.30	....	0.10	2.30	....	....
28	....	....	....	....	....	0.40
29	....	....	....	....	....	....
30	....	....	0.12	....	1.65	....
31	....	....	....	....	0.25	....
Total....	0.71	2.88	2.97	10.06	4.41	9.18

natural low position and an extremely high water-holding capacity, will be much more easily affected adversely than the lighter and more porous ones. This is clearly borne out by the data given in table 3 which were obtained from the 1928 spring potato crop.

In preparing the data in table 3, only the results from those fields where the soil was uniformly of one type were used. There is a wide variation in the total

acreages listed for the different soil types which naturally will affect the final results to some extent, but which may enable one to get some idea of the differences in the response of some of the soil types to potato production during the spring of 1928 as compared with that of the spring and fall of 1927.

It is indeed interesting to note that the Norfolk and Blanton soils compared very favorably with the Portsmouth and St. John's loamy fine sand soils as potato soils during this season and were superior to Leon fine sand, loamy phase. On first thought it may appear difficult to explain this peculiarity in light of the results reported in tables 1 and 2. However, an examination of the rainfall data in tables 4 and 5 will reveal the proper explanation.

Table 4 shows the average monthly rainfall in inches for this area from 1876 to 1926. The average rainfall for January, February, March, and April, the spring potato season, is very light. The same is true for October, November, and December, the fall potato season. The rainfall for 1927 was about average.

The figures in table 5, however, reveal a different situation. The rainfall during the spring potato season of 1928 was much above the average.

In view of the fact that the inability of the lighter soils to retain sufficient moisture for maximum potato production during an average season is responsible in large measure for their inferiority as potato soils, the above rainfall data reveal a very satisfactory explanation for the peculiar reversal of form of some of the soil types to potato production during the spring of 1928 over the spring and fall of 1927.

Beginning with the last week in January, the rainfall for the most part was about as near ideal for potato growing as could be expected until the last half of April when the unprecedented sum of 10.06 inches fell from April 10 to 27, inclusive. Until the heavy rainfall occurred during the last half of April the potatoes on the Portsmouth and St. John's soils had a decided lead on those on the lighter, drier types of soil, but even at that the potatoes were doing well on the latter types because of the very favorable rainfall distribution. However, it was during the period of heavy rain in April that the potatoes on the heavy Portsmouth and St. John's soils suffered severely from excessive rainfall, while at the same time the Blanton and Norfolk soils were in excellent condition for optimum potato production. Satisfactory drainage was much more difficult to obtain on the St. John's than on the Portsmouth soils during this period. However, potatoes on both types suffered severely in places. The high water-holding capacity of the St. John's soil, together with its natural low position, made it next to impossible to drain it sufficiently during the heavy rains in April to prevent considerable damage to the crop. During this same period the potatoes on the Blanton and Norfolk soils were being very highly favored by an adequate water supply.

However, in spite of the serious handicap placed on the Portsmouth and St. John's soils by the heavy rainfall in April, the Portsmouth still maintained its lead as a potato-producing soil with St. John's following only slightly behind Blanton, which in turn rated very close to Portsmouth during this season.

The Norfolk soil made a very creditable showing during this period also. In addition to being favored with a satisfactory rainfall distribution, this soil had another decided advantage over the two previous seasons because of the fact that nothing but the flat phase of Norfolk fine sand was planted to potatoes this season, whereas during the two previous seasons considerable Norfolk fine sand was planted. The Norfolk fine sand is much inferior to the flat phase of Norfolk fine sand as a potato-producing soil, and tends to lower the average yield. The flat phase of Norfolk fine sand normally rates very close to Blanton fine sand as a potato soil.

The average acre yield of the Leon fine sand, loamy phase, was somewhat below that of Norfolk fine sand, flat phase, and Blanton fine sand during this season. This is due largely to the fact that areas of the fomer contain pockets of hardpan which prevent water passing through the subsoil fast enough to prevent the plants from being seriously damaged by excessive water during periods of excessive rainfall.

The response of other crops to different soil types is just as pronounced as that with potatoes. This may readily be seen from a study of the discussion and photographs which follow.

Notwithstanding the fact that Blanton fine sand proved to be only a mediocre potato soil it is an excellent soil for grapes. This is clearly borne out by plate 1, figure 1. These plants were set March 4, 1927, and the picture was taken July 21 of the same year. Loamy phase of Leon fine sand had about the same value as a potato-producing soil as Blanton but is far inferior to Blanton as a grape soil. This is clearly brought out by a comparison of figures 1 and 2, plate 1. The grapes shown in figure 2 were set the same time as those in figure 1 and the picture was taken on the same day. The cultural treatments for the two vineyards were as nearly the same as possible. The difference in vine growth is due almost wholly to soil.

Since Blanton fine sand and loamy phase of Leon fine sand had almost equal values as potato soils, at first thought it may seem difficult to explain the difference in their reaction to grapes. However, upon a more careful consideration of the characteristics of these two types of soil as related to the development of the root system of the grape vine one may easily account for this difference Both of these soils proved to be somewhat droughty, or poor at retaining. moisture during an average season, for a first class potato soil. This is due in part, at least, to the fact that the potato crop is shallow rooted and is grown during the season of light rainfall which is not properly distributed to keep the moisture content of these soils right for optimum potato growth. The grape vine has a much more rangy root system than the potato plant and grows through the wet summer season as well as the drier potato season. The nature of the Blanton soil is such that the water table during the period of heavy rainfall in the summer months seldom gets nearer the surface than 4 to 5 feet, whereas with the loamy phase of Leon fine sand it is customary for the water table to rise to within 1½ to 2 feet of the surface of the ground. This means, of course, that

acreages listed for the different soil types which naturally will affect the final results to some extent, but which may enable one to get some idea of the differences in the response of some of the soil types to potato production during the spring of 1928 as compared with that of the spring and fall of 1927.

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The figures in table 5, however, reveal a different situation. The rainfall during the spring potato season of 1928 was much above the average.

In view of the fact that the inability of the lighter soils to retain sufficient moisture for maximum potato production during an average season is responsible in large measure for their inferiority as potato soils, the above rainfall data reveal a very satisfactory explanation for the peculiar reversal of form of some of the soil types to potato production during the spring of 1928 over the spring and fall of 1927.

Beginning with the last week in January, the rainfall for the most part was about as near ideal for potato growing as could be expected until the last half of April when the unprecedented sum of 10.06 inches fell from April 10 to 27, inclusive. Until the heavy rainfall occurred during the last half of April the potatoes on the Portsmouth and St. John's soils had a decided lead on those on the lighter, drier types of soil, but even at that the potatoes were doing well on the latter types because of the very favorable rainfall distribution. However, it was during the period of heavy rain in April that the potatoes on the heavy Portsmouth and St. John's soils suffered severely from excessive rainfall, while at the same time the Blanton and Norfolk soils were in excellent condition for optimum potato production. Satisfactory drainage was much more difficult to obtain on the St. John's than on the Portsmouth soils during this period. However, potatoes on both types suffered severely in places. The high water-holding capacity of the St. John's soil, together with its natural low position, made it next to impossible to drain it sufficiently during the heavy rains in April to prevent considerable damage to the crop. During this same period the potatoes on the Blanton and Norfolk soils were being very highly favored by an adequate water supply.

However, in spite of the serious handicap placed on the Portsmouth and St. John's soils by the heavy rainfall in April, the Portsmouth still maintained its lead as a potato-producing soil with St. John's following only slightly behind Blanton, which in turn rated very close to Portsmouth during this season.

The Norfolk soil made a very creditable showing during this period also. In addition to being favored with a satisfactory rainfall distribution, this soil had another decided advantage over the two previous seasons because of the fact that nothing but the flat phase of Norfolk fine sand was planted to potatoes this season, whereas during the two previous seasons considerable Norfolk fine sand was planted. The Norfolk fine sand is much inferior to the flat phase of Norfolk fine sand as a potato-producing soil, and tends to lower the average yield. The flat phase of Norfolk fine sand normally rates very close to Blanton fine sand as a potato soil.

The average acre yield of the Leon fine sand, loamy phase, was somewhat below that of Norfolk fine sand, flat phase, and Blanton fine sand during this season. This is due largely to the fact that areas of the fomer contain pockets of hardpan which prevent water passing through the subsoil fast enough to prevent the plants from being seriously damaged by excessive water during periods of excessive rainfall.

The response of other crops to different soil types is just as pronounced as that with potatoes. This may readily be seen from a study of the discussion and photographs which follow.

Notwithstanding the fact that Blanton fine sand proved to be only a mediocre potato soil it is an excellent soil for grapes. This is clearly borne out by plate 1, figure 1. These plants were set March 4, 1927, and the picture was taken July 21 of the same year. Loamy phase of Leon fine sand had about the same value as a potato-producing soil as Blanton but is far inferior to Blanton as a grape soil. This is clearly brought out by a comparison of figures 1 and 2, plate 1. The grapes shown in figure 2 were set the same time as those in figure 1 and the picture was taken on the same day. The cultural treatments for the two vineyards were as nearly the same as possible. The difference in vine growth is due almost wholly to soil.

Since Blanton fine sand and loamy phase of Leon fine sand had almost equal values as potato soils, at first thought it may seem difficult to explain the difference in their reaction to grapes. However, upon a more careful consideration of the characteristics of these two types of soil as related to the development of the root system of the grape vine one may easily account for this difference Both of these soils proved to be somewhat droughty, or poor at retaining. moisture during an average season, for a first class potato soil. This is due in part, at least, to the fact that the potato crop is shallow rooted and is grown during the season of light rainfall which is not properly distributed to keep the moisture content of these soils right for optimum potato growth. The grape vine has a much more rangy root system than the potato plant and grows through the wet summer season as well as the drier potato season. The nature of the Blanton soil is such that the water table during the period of heavy rainfall in the summer months seldom gets nearer the surface than 4 to 5 feet, whereas with the loamy phase of Leon fine sand it is customary for the water table to rise to within $1\frac{1}{2}$ to 2 feet of the surface of the ground. This means, of course, that

the roots of the grape vine on the Blanton soil have a feeding range down to 4 to 5 feet during the wettest season of the year whereas on the loamy phase of Leon fine sand it can feed only from $1\frac{1}{2}$ to 2 feet deep. Roots penetrating the soil to a greater depth than $1\frac{1}{2}$ to 2 feet on this type of soil during the dry weather are automatically killed by excessive water during the wet summer season. As a result the plants are severly stinted and very often killed outright on the loamy phase of Leon fine sand whereas they are not injured on the Blanton soil.

Blanton soil is also very well adapted to the production of pecans, plums, peaches, and pears as may readily be seen from plate 2, figure 1. The same crops make a compete failure, however, when set on loamy phase of Leon fine sand, Leon fine sand, and St. John's loamy fine sand. This is clearly brought out in plate 2, figure 2. The trees in these two groves were set early in March, 1927, by the same group of men and were treated as nearly alike as was humanly possible. The photographs were taken on July 13 of the same year with the results shown.

Blanton fine sand is a good soil for pecans, plums, peaches and pears for the same reason that it is good for grapes, whereas the loamy phase of Leon fine sand is a poor soil for these crops for the same reason that it is not adapted to the production of grapes.

St. John's loamy fine sand proved to be a very satisfactory soil for potatoes but an extremely poor one for the other crops mentioned. It is a good potato soil largely because it retains moisture well during the potato growing season and is capable of taking care of the moisture needs of the potato crop. On the other hand, it has a water table within from 6 to 9 inches of the surface during the wet summer season, which makes it practically impossible to grow deep rooted crops unless the drainage is such that the water table can be lowered sufficiently to meet their needs. The characteristic low position of this type of soil makes it almost impossible for the water table to be lowered sufficiently to enable these crops to grow.

Blanton fine sand also produces blueberries satisfactorily whereas Leon fine sand makes a complete failure with the same crop. This is clearly brought out in plate 3. The plants were all set out the same time and received similar treatment. The plants in the foreground are on Leon fine sand and those in the background on Blanton fine sand.

Blanton fine sand is a satisfactory soil for blueberries for the same reason it is suited to grapes. Because of its peculiar make-up, the Leon fine sand is decidedly unfit, not only for blueberries but for practically all other crops, unless there is absolute water control practiced. This soil type is characterized by a compact, brownish, organic, hardpan layer ranging from 12 to 18 inches beneath the surface. This hardpan layer is impervious to water and the roots of many cultivated plants, and varies from 1 to several inches in thickness, usually averaging about 3 to 4 inches thick. The soil above it is very coarse and poor at retaining capillary moisture. During periods of low rainfall the small amount of capillary moisture in the soil above the hardpan layer is

quickly exhausted by plants and can not be replenished by capillary movement from below. The plants naturally die from lack of water. On the other hand, during periods of heavy rainfall the surplus water is unable to percolate through this hardpan layer and is forced to accumulate as free water above this layer, thus drowning out whatever crops may have been growing up to that time.

The roots of most cultivated crops are unable to penetrate this peculiar hardpan. However, should they be able to penetrate it they would encounter free water immediately beneath it and further growth would be checked.

The same striking contrast may be had with tung oil trees when planted on Norfolk fine sand and Leon fine sand. This is clearly shown in plate 4, figure 1. At the same time that blueberries, grapes, pecans and tung oil fail on Leon fine sand crotalaria apparently does well. This is shown by plate 4, figure 2.

Norfolk fine sand is somewhat more droughty than Blanton fine sand and also has a lower water table. Ordinarily, however, Norfolk fine sand contains enough moisture during the dry season to meet the requirements of the tung oil tree and has a water table low enough during the wet summer season so as not to interfere with the normal root development of that plant. The tung oil tree fails to make good on Leon fine sand for the same reason that blueberries and most other crops do.

Apparently crotalaria is able to adjust itself successfully to Leon fine sand under some conditions, at least, a fact brought out in plate 4, figure 2. The roots of the crotalaria were able in this case to penetrate the hardpan layer.

Corn is just as sensitive to soil types as any of the other crops mentioned. This is very clearly brought out by plate 5. The corn in figure 2 was planted about one week earlier than that in figure 1. The cultural treatments were practically the same for the two fields. The pictures were taken on July 13, 1927. The corn in figure 1 was planted on Portsmouth loamy fine sand and that in figure 2 on Leon fine sand.

Corn is adapted to Portsmouth loamy fine sand for the same reason that potatoes are adapted to it. It is also unadapted to Leon fine sand for the same reason that most other crops are adapted to it.

Notwithstanding the fact that grapes, pecans, plums, pears, peaches, and many other crops make a complete failure on St. John's loamy fine sand, peppers appear to be perfectly at home on this soil. This is clearly evident from plate 6, figure 1. This soil also rated well as a potato soil. It is equally well adapted to bulbs, onions, peppermint, and numerous other truck crops. On the other hand there are several crops, other than those mentioned, which are wholly unsuited to this soil.

Pepper, bulbs, onions, peppermint, and such crops are well adapted to St. Johns loamy fine sand soil for the same reason that potatoes are. However, those crops with a rangy root system are entirely unsuited to this type of soil.

Notwithstanding the fact that Portsmouth loamy fine sand is very poorly adapted to such crops as grapes, peaches, plums, pears, tung oil and many

others, it is very well suited to the production of Japanese sugar cane, as evidenced by plate 6, figure 2.

Portsmouth loamy fine sand retains moisture well during the dry seasons. Consequently it is well suited to the production of Japanese sugar cane which has a shallow root system and requires an enormous amount of water during the growing season. During the wet summer season the water table on this soil is within 12 to 18 inches of the surface, a condition which makes it entirely unsuited to the production of crops with a rangy root system.

Loamy phase of Leon fine sand soil rates as only a mediocre Irish potato soil, is a very poor soil for grapes, pecans, and several other crops but is very good as a sweet potato soil. The potatoes shown in plate 7, figure 1 all came from one hill of potatoes grown on loamy phase of Leon fine sand. This soil happens to possess the particular peculiarities necessary for a satisfactory sweet potato soil.

That peppermint is just as sensitive to a change in soil type as most of the other crops discussed, is clearly shown by plate 7, figure 2. The plants in the right foreground are on Cypress pond soil whereas those in the left background are on loamy phase of Leon fine sand. The last named soil is entirely too droughty in character to be a suitable soil for the production of peppermint whereas the Cypress pond soil contains the proper constituents to enable it to hold suitable moisture for a satisfactory growth of peppermint.

The data and photographs in this article make up only a small proportion of the total information collected on the point under discussion. However, these data are sufficiently conclusive to indicate the value of soil surveys in the economic development of Florida soils.

PLATE 1

GRAPE PLANTS SET MARCH 4, 1927, PHOTOGRAPHED JULY 21, 1927

FIG. 1. Grapes on Blanton fine sand.
FIG. 2. Grapes on Leon fine sand (loamy phase).

Fig. 1

Fig. 2

PLATE 2

PLANTS SET IN EARLY MARCH, 1927, PHOTOGRAPHED JULY 13, 1927

FIG. 1. Peaches, pears, and plums on Blanton fine sand.
FIG. 2. Pecans on Leon fine sand (loamy phase).

Fig. 1

Fig. 2

PLATE 3

BLUEBERRIES; FOREGROUND LEON FINE SAND; BACKGROUND BLANTON FINE SAND

Plants all set at the same time and cultivated uniformly throughout

PLATE 4

Fig. 1. Tung Oil trees; left, Norfolk fine sand; right, Leon fine sand. Plants all set at the same time and cultivated uniformly throughout.

Fig. 2. Crotalaria on Leon fine sand. Photographed November 2, 1927.

FIG. 1

FIG. 2

PLATE 5

FIG. 1. Corn on Portsmouth Loamy fine sand. Photographed July 13, 1927.
FIG. 2. Corn on Leon fine sand. Photographed July 13, 1927.

FIG. 1

FIG. 2

PLATE 6

Fig. 1. Peppers on St. John's loamy fine sand. Plants set in April, 1927, photographed July 13, 1927.

Fig. 2. Japanese sugar cane on Portsmouth loamy fine sand. Photographed November 5, 1927, produced 40 tons to the acre.

FIG. 1

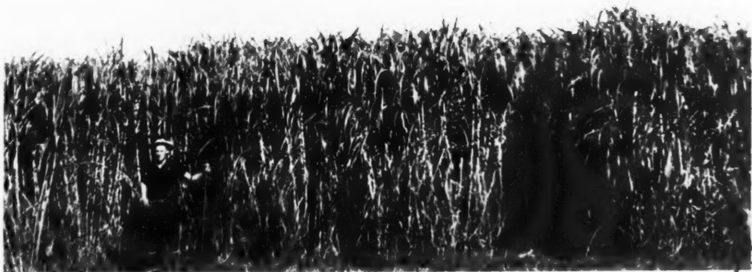

FIG. 2

PLATE 7

FIG. 1. Sweet potatoes grown on Leon fine sand, loamy phase. All from one hill.

FIG. 2. Peppermint; foreground, Cypress Pond soil; left background, Leon fine sand, loamy phase. Photographed July 13, 1927.

FIG. 1

FIG. 2

THE USE OF DYES IN THE ISOLATION OF A NITRITE OXIDIZING ORGANISM[1]

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Although the process of nitrification in its many different aspects has been the subject of a great deal of study since the first observations of Schloessing and Müntz in 1878, the isolation of the nitrifying organisms still remains a difficult task in the hands of most laboratory workers. These bacteria are obligate autotropic organisms possessing definite physiological characteristics which differentiate them from other bacteria. They develop in a pure mineral medium containing oxidizable inorganic substances which serve as the only source of energy. Organic nutrients are not necessary for growth or energy requirements. Such nutrients have been shown by numerous workers to exert a toxic effect on the nitrifying bacteria, especially when used in the amounts that are commonly employed in bacteriological media. By means of the energy secured from the oxidation of the inorganic nitrogenous compounds these organisms are able to reduce carbon dioxide, thus securing carbon from this source for the synthesis of their organic structures.

Advantage is taken of these physiological characteristics in isolating the nitrifying bacteria. By the use of an inorganic medium containing the desired oxidizable nitrogenous compound it is possible to eliminate from cultures of these organisms the majority of the soil flora, especially the obligate heterotropic bacteria. It is an established fact, however, that certain organisms which are non-oxidizers of ammonium and nitrite compounds are capable of rapid growth in these highly specialized media.

HISTORICAL

Winogradsky (14) first reported the isolation of the nitrifying bacteria in 1890. Since that time many investigators have attempted to secure pure cultures of these organisms by following the technique developed by Winogradsky. A few of these workers have been successful in their efforts. Others have failed because of their inability to eliminate certain contaminating forms. In numerous instances investigators working with the nitrifying bacteria have proceeded with impure cultures because of the difficulties arising in attempting to isolate these organisms in pure cultures. Meek and Lipman (10), studying

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the relation of reaction and salt concentration of the medium on the nitrifying bacteria, used crude cultures. They make the following statement in regard to the use of such cultures: "Difficulties encountered in attempts to obtain pure cultures of the nitrifying bacteria led us to adopt the use of a crude, but invigorated culture in each case, which was produced by numerous successive transfers to fresh media from a strong soil culture in the solutions above described." Gowda (8) used cultures of the nitrifying organisms which had gone through a number of sub-cultures. He made no attempt to secure pure cultures of these organisms.

It is evident that further work is needed to develop a more desirable technique for the isolation of these bacteria than exists at the present time. This would permit studies to be made which would assist in the clarification of certain conceptions pertaining to the morphological and physiological characteristics of the nitrifying bacteria. The work reported in this paper is confined to a study of the isolation of a nitrite oxidizing organism and a discussion of the predominating contaminating forms.

The well-known fact that certain dyes exhibit a selective action toward various organisms suggested their use in this study as a means of freeing cultures of the nitrite oxidizing organism of bacteria that persist when other methods are used. The bacteriostatic action of dyes, especially the dyes of the tri-phenyl methane group, has received considerable attention. Churchman (4) demonstrated rather conclusively the bacteriostatic action of gentian violet toward cultures of *B. anthracis*. In a more recent study Churchman (5) has shown acid fuchsin to possess a bacteriostatic action the reverse of that possessed by gentian violet. Anderson (1), Batchelor and Curie (2), and Vandecaveye (12) have made use of dyes in the isolation of the root nodule bacteria of legumes.

METHODS

The first step in the isolation of the nitrite oxidizing organism is the securing of a crude culture of the organism which is comparatively free from other bacterial forms. This is accomplished by the enrichment process: i.e., the development of the organism in a medium suited to its particular requirements. Successive transfers to such a medium with the subsequent additions of nitrite after oxidation tend to increase the numbers of this organism accompanied by a decrease in the number of undesirable types. Many investigators have carried such cultures through numerous transfers with the hope of finally eliminating all contaminating forms, thereby securing a pure culture. This, however, has not proved successful. Gibbs (7) has shown these contaminating forms to persist after 35 successive transfers. Gowda (9) was unable to secure pure cultures by this method. He found certain contaminating forms to be present after many successive transfers.

The media used in the cultivation of the nitrite oxidizing organism were the same as those used by Gibbs. These media were prepared with conductivity

water and chemicals of the highest purity and were of the following composition:

Liquid medium

	gm.
Sodium nitrite	1.0
Sodium carbonate	1.0
Di-potassium phosphate	0.5
Sodium chloride	0.5
Magnesium sulfate	0.3
Ferric sulfate	Trace
Water	1000.0

Washed agar medium

1 cc. portions of each of the following solutions were added to 15 cc. of a 2 per cent washed agar:

		gm.
(a)	Di-potassium phosphate	1.5
	Water	100.0
(b)	Sodium nitrite	1.5
	Sodium carbonate	1.5
	Water	100.0
(c)	Magnesium sulfate	0.45
	Sodium chloride	0.75
	Ferric sulfate	0.02
	Water	100.0

Twenty-five-cubic-centimeter portions of the liquid medium were dispensed into 200-cc. Erlenmeyer flasks and sterilized in the autoclave. The solutions used with the washed agar were prepared and sterilized separately. These were mixed with the washed agar at the time of pouring plates. One-cubic-centimeter portions of solution (b) were used as enrichment additions to cultures which had oxidized the nitrite.

A portion of soil, approximately 2 gm. in weight, was inoculated into a flask of the nitrite medium and incubated at 28°C. Oxidation of the nitrite usually was complete after a period of from 15 to 20 days. This was determined by the use of Tromsdorff's reagent. A negative test for nitrite indicated that the nitrite had been oxidized to nitrate. One loop of this actively oxidizing culture was transferred to a 25-cc. portion of sterile nitrite medium. When oxidation was complete another transfer was made. This process was continued until four successive transfers of the culture had been made. Nitrite was added to this culture at intervals of five to seven days, depending upon the rate of oxidation. At this stage the more common soil forms had been eliminated, but the culture was still impure as indicated by growth on plain agar plates.

Plain agar loop dilution plates made from this actively oxidizing culture showed the presence of numerous colonies of two types of organisms. One

organism was a gram-negative coccus, usually occurring in pairs and forming a small circular pink colony. The other organism was a small gram-negative, extremely motile bacillus. This organism formed a small circular yellow

TABLE 1

Effect of dyes on a crude culture of the nitrite oxidizing organism

	DYE USED	MINUTES OF EXPOSURE								
		1	2	3	5	7	10	15	20	30
Chemical group	Phenyl-methane:									
	Acid green 1.0 per cent	+	+	+	+	+	+	+	+	+
	Acid green 1.5 per cent	+	+	+	+	+	+	+	+	+
	Malachite green 0.5 per cent	+	+	−	+	+	−	−	−	−
	Malachite green 1.5 per cent	−	−	−	−	−	−	−	−	−
	Brilliant green 1.0 per cent	−	−	−	−	−	−	−	−	−
	Acid fuchsin 1.5 per cent	+	+	+	+	+	+	+	+	+
	Basic fuchsin 1.0 per cent	+	+	+	+	+	+	+	+	+
	Crystal violet 0.5 per cent	−	−	−	−	−	−	−	−	−
	Methyl green 0.125 per cent	−	−	−	−	−	−	−	−	−
	Methyl violet 0.5 per cent	−	−	−	−	−	−	−	−	−
	Rosaniline hydrochloride 1.0 per cent	+	+	+	+	+	+	+	+	+
	Xanthene:									
	Eosin 1.0 per cent	+	+	+	+	+	+	+	+	+
	Eosin B. 1.0 per cent	+	+	+	+	+	+	+	+	+
	Rose bengal 0.75 per cent	+	+	+	+	+	+	+	+	+
	Rose bengal 1.0 per cent	−	−	−	−	−	−	−	−	−
	Quinone-imide:									
	Methylene blue 1.0 per cent	−	−	−	−	−	−	−	−	−
	Methylene blue 0.5 per cent	+	+	−	+	+	−	+	−	−
	Thionine 1.0 per cent	+	+	+	−	−	−	−	−	−
	Neutral red 1.0 per cent	+	0	+	0	+	0	+	0	0
	Neutral red 1.5 per cent	+	+	+	+	+	+	+	+	+
	Azo:									
	Bismarck brown 1.0 per cent	+	+	+	+	+	+	+	+	+
	Orange G. 1.0 per cent	+	+	+	+	+	+	+	+	+
	Control	Oxidized								

+Nitrite oxidized to nitrate.
−Nitrite not oxidized.
0 Not determined.

colony. A more detailed description of these organisms will be found later in this paper.

An experiment, preliminary in nature, was designed to test the effect of the dyes upon this impure culture. Two per cent aqueous solutions of dyes were prepared and designated as stock dyes. Solutions of the desired strength

were prepared from these stock dyes. The technique of the test consisted of adding four drops of the culture to 4 cc. of the dye solution in a test tube. The dye solution and culture were thoroughly mixed and one drop transferred to a flask of nitrite medium after intervals of 1, 2, 3, 5, 7, 10, 15, 20, and 30 minutes of exposure. To serve as a control four drops of the actively oxidizing culture were added to 4 cc. of sterile water. After mixing, one drop was transferred

TABLE 2

Growth in plain broth of cultures oxidizing nitrite

	DYE USED	MINUTES OF EXPOSURE								
		1	2	3	5	7	10	15	20	30
Chemical group	Phenyl-methane:									
	Acid green 1.0 per cent	+	+	+	+	+	+	+	+	+
	Acid green 1.5 per cent	+	+	+	+	+	+	+	+	+
	Malachite green 0.5 per cent	–	–	0	–	–	0	0	0	0
	Acid fuchsin 1.5 per cent	+	+	+	+	+	+	+	+	+
	Basic fuchsin 1.0 per cent	+	+	+	+	+	+	+	+	+
	Rosaniline hydrochloride 1.0 per cent	+	–	–	–	–	–	–	–	–
	Xanthene:									
	Eosin 1.0 per cent	+	+	+	+	+	+	+	+	+
	Eosin B. 1.0 per cent	+	+	+	+	+	+	+	+	+
	Rose bengal 0.75 per cent	+	+	+	+	+	+	+	+	+
	Quinone-imide:									
	Methylene blue 0.5 per cent	+	+	0	+	+	0	+	0	0
	Thionine 1.0 per cent	+	+	–	0	0	0	0	0	0
	Neutral red 1.0 per cent	+	0	+	0	+	0	+	0	0
	Neutral red 1.5 per cent	+	+	+	+	+	+	+	+	+
	Azo:									
	Orange G. 1.0 per cent	+	+	+	+	+	+	+	+	+

to a flask of sterile nitrite medium. All flasks were incubated at 28°C. and tested for nitrite at intervals of four to five days during a 30-day incubation period.

RESULTS OF INVESTIGATION

Effect of dyes on oxidation

Table 1 shows the dye used, the time of exposure, and the relative bactericidal effect of the dye on the nitrite oxidizing organism, as determined by the inhibition of nitrate formation.

From table 1 it will be noted that a number of the dyes, in the concentrations used, inhibited the development of the nitrite oxidizing organism as indicated by the failure of the culture to oxidize nitrite.

Broth cultures

In the foregoing experiment all of the dye cultures which failed to show oxidation were discarded. Those which showed oxidation were transferred to plain broth in order to determine whether organisms were present which were capable of growing in that medium. According to Winogradsky the criterion of purity of the nitrifying bacteria is the ability to oxidize nitrite and the inability to develop in plain broth. The results of this experiment are shown in table 2.

The cultures which showed no growth when transferred to plain broth; namely, the cultures exposed to malachite green 0.5 per cent for 1, 2, 5, and 7 minutes; rosaniline hydrochloride 1.0 per cent for 2, 3, 5, 7, 10, 15, 20, and 30 minutes; and thionine 1.0 per cent for 3 minutes, fulfilled the criterion for purity as put forth by Winogradsky in his early studies of the nitrifying bacteria.

Plain agar plate cultures

It seemed desirable, however, to subject these cultures to a further test, especially one in which aerobic conditions would be at a maximum. For this purpose plain agar plates were used. These plates were streaked by the use of an inoculating machine as described by Varney (13). This method consists of applying the inoculum to the surface of the medium while the plate is rapidly rotating. This technique proved very desirable and many well-isolated colonies arranged in concentrics about the plate were secured. These plates were placed under a bell jar to prevent excessive evaporation during incubation. After a period of 15 days many very small white colonies, which were almost invisible to the naked eye, appeared on the surface of the medium. These colonies were examined under the low power objective of the microscope and were found to be all of the same type. On plain agar plates they varied from very small to 0.15 mm. in diameter. The centers of the colonies appeared quite dark with a gradual fading to the edge, which was irregular and lacerated. The organisms of these colonies stained readily with the ordinary bacteriological dyes, being stained especially well with cold carbol fuchsin when applied for only a few seconds. When stained by Gram's method they retained this stain. Carbol fuchsin stained preparations showed these organisms to possess a flagellum-like attachment at one pole, which in many instances was several times as long as the body of the cell. Colonies of this organism when transferred to a nitrite medium failed to produce oxidation of the nitrite.

Washed agar plate cultures

Washed agar plates were inoculated from the same cultures in a similar manner and incubated under a bell jar at a temperature of 28°C. After about 15 days many very small white colonies were visible to the naked eye. When

examined under the low power objective of the microscope two distinct types of colonies were observed, one type being morphologically identical with the colonies which developed on the plain agar plates, while the other type was at first rather irregular in shape, but as the colony grew larger and older it became round or oval and possessed a smooth entire edge and a finely granular internal structure. Under the microscope, colonies of the latter type appeared as light brown colonies and varied from extremely small to 0.2 mm. in diameter. On all washed agar plate cultures containing colonies of this type oxidation of the nitrite took place.

Nitrite oxidizing organism

Cold carbol fuchsin applied for only a few seconds proved to be the most desirable stain for demonstrating the morphological characteristics of the nitrite oxidizing organism. In stained preparations it occurs singly, in pairs, and in irregular clusters. It stains evenly, varies from oval to spherical in shape, the majority of the cells being spherical, and is from 0.8 to 1.0μ in diameter. It is non-motile and retains Gram's stain when stained by that method. The morphology of the nitrite oxidizing organism is such that it is rather difficult to determine whether it should be classed as a coccus or as a rod form.

Separation of organisms on washed agar plate cultures

The two types of colonies appearing on washed agar plates were so small and so numerous that it was necessary to resort to the use of a modified Barber pipette in order to obtain pure cultures of these organisms. This was carried out in the following manner. Portions of agar were removed from the petri dish to a sterile cover slip, which was then inverted and supported under the low power objective of the microscope in such a manner that individual colonies could be examined. Colonies were selected and fished from the surface of the medium by the aid of a mechanically operated sterile glass pipette inserted from underneath the inverted colonies and slowly raised until contact with the desired colony was made. By this means pure cultures of each type of organism were secured and grown both in liquid and on solid media.

Oxidation of ammonia and nitrite

Throughout the course of this study a great many colonies of both types were transferred to nitrite agar and to a nitrite liquid medium. In almost all cases cultures originating from colonies possessing the smooth entire edge were capable of nitrite oxidation, while the cultures developing from the other type of colony, although growth from these transfers was quite evident as determined by microscopic and macroscopic examination, were not capable of producing nitrate from nitrite. It was thought desirable to test the ability of these two organisms to oxidize ammonium compounds to nitrite. Accordingly

numerous colonies of each organism were transferred to flasks of a sterile medium containing oxidizable nitrogen in the form of ammonium sulfate. After from 20 to 30 days microscopic examinations and chemical tests for the presence of ammonia and nitrite were made. In no case did cultures of either organism oxidize the ammonium compound to compounds of nitrite. Microscopic examinations of the cultures originating from the irregular type of colonies revealed the presence of large numbers of organisms in the ammonium medium. The fact that this organism grew very abundantly in both the ammonium and nitrite media without the oxidation of these compounds is sufficient evidence to eliminate it as belonging to the nitrifying group of bacteria.

Non-nitrite oxidizing organism

In numerous instances the polar flagellum-like attachment, which was noted when this organism was stained with cold carbol fuchsin, appeared to join a smaller deeply stained body to the bacterial cell. The length of this attachment varied on different cells. On many cells it was from 7 to 10μ long, whereas on others it was much shorter. In some preparations the organisms were clustered together with the flagella-like attachments radiating away from the center of the cluster of bacteria. In many instances the cells were unevenly stained, there being an area at one end of the cell, sometimes near the flagellum-like attachment and sometimes at the other end, which would not take the stain. These cells were generally oval and somewhat pointed at the ends. They varied from 0.5 to 1.0μ in width and from 1.2 to 1.8μ in length. Among the early investigators who observed in their cultures an organism of similar description were Stutzer and Hartleb (11). They gave the name *Hypomicrobium* to this non-nitrifying organism. The greatest morphological difference these investigators found between *Nitrobacter* and *Hypomicrobium* was the thread-like growth from one pole of the *Hypomicrobium*.

Gibbs noticed a very short stem-like growth in many stained preparations of his pure cultures of *Nitrobacter*. Such growth was very noticeable when the preparation was treated by any method of staining flagella, but was seldom seen in the ordinary stained preparations. Gibbs found surface colonies on washed agar plates to occasionally appear regular in outline but more often irregular and spreading. This organism was distinctly oval in shape, commonly found in single cells or in pairs, about 0.6 to 0.8μ wide and 1.0 to 1.2μ long. Washed agar surface colonies of the nitrite oxidizing organism isolated in this study differ to some extent from washed agar colonies of the organism described by Gibbs. In almost all cases they appeared after 15 to 20 days as regular round or oval colonies possessing an entire edge and a finely granular internal structure. This was especially true in the case of well-isolated colonies but on thickly seeded plates there was a greater tendency toward irregular colonies.

Fred and Davenport (6) isolated and described an organism which appears

to be morphologically identical with the non-nitrifying organism isolated and described in this study. They found this organism to be capable of oxidizing nitrite to nitrate. They refer to it as *Nitrobacter*. In stained preparations they found this organism to possess a flagellum-like attachment which could be demonstrated when the organism was stained with cold carbol fuchsin or with Loeffler's flagella stain. One of the most characteristic arrangements in stained preparations was that of clumps of zooglea-like masses with only a few loose cells in the field. Some mounts showed the cells scattered more or less evenly over the field. They found the cells to stain unevenly, the center or more often one end of the cell being well stained, while the remainder, except the outline of the cell wall, did not take the stain at all. The microscopic appearance of stained preparations of the non-nitrifying organisms encountered in this study was characteristic in all details with the photomicrographs prepared and published by these investigators. The non-nitrifying organism of this study was found rather often in such a characteristic grouping as illustrated by a photomicrograph and a drawing in the paper of Fred and Davenport.

Fred and Davenport gave no detailed colony characteristics of the organism they have designated as *Nitrobacter*. From the rather striking morphological differences of the organism designated by Gibbs as being *Nitrobacter* and the organism described by Fred and Davenport, especially in the light of this study, it seems quite probable that these investigators were dealing with two different organisms.

Terminology used

In this study it has not seemed desirable to use the rather common term, *Nitrobacter*, in reference to the nitrite oxidizing organism. Neither has it appeared desirable, at this time, to classify this organism according to the terminology used in Bergey's *Manual of Determinative Bacteriology* (3) for those organisms capable of nitrite oxidation. Sufficient evidence has not been secured in this study definitely to classify this organism as being *Nitrobacter winogradskyi* Buchanan, or as being *Nitrosococcus nitrosus* (Migula) Bergey et al. For these reasons reference has been made to this organism throughout this paper as "the nitrite oxidizing organism."

Further experiments

Experiments testing the effect of malachite green, thionine, and rosaniline hydrochloride on crude cultures of the nitrite oxidizing organism were repeated many times. The results with malachite green and thionine were not generally favorable, as these dyes did not always check the growth of the contaminating forms. In numerous cases these forms were found to resist greater concentrations of the dyes than did the nitrite oxidizing organism. Very satisfactory results were usually obtained with the use of rosaniline hydrochloride, a tri-phenyl methane derivative. This dye proved to be a very satisfactory agent in eliminating the two most objectionable contaminating

forms, especially when used in 1.0 per cent concentrations and with exposures varying from 5 to 30 minutes.

Contaminating forms

In this study three contaminating bacterial forms were encountered, one of which has been described in detail. The other two organisms were found to grow very rapidly in the inorganic medium which was employed in this study as well as in ordinary nutrient media. The description of these organisms is as follows:

COCCUS FORM

Morphology. In 24-hour cultures on agar slants this organism appears as a coccus and is usually found in pairs. It varies from 0.8 to 1.0μ in diameter. It is non-motile, non-spore forming, and reacts negatively when stained by Gram's method. It stains readily with the ordinary dyes and can best be studied when stained with carbol fuchsin for about 30 seconds.

Cultural characteristics. Growth on plain agar slants is smooth, raised, and characterized by a glistening surface. Agar plate surface colonies are circular, raised, finely granular, and possess an entire edge. Surface colonies may develop to as large as 2 or 3 mm. in diameter. Colonies within the medium assume a lens-shaped appearance. Both surface and deep colonies on forming are orange, and become pink as the colony ages.

Physiology. This organism grows well on the surface of nutrient agar, on gelatin, and in nutrient broth. It does not liquefy gelatin, reduce nitrate, nor possess diastatic properties. Litmus milk becomes alkaline in reaction after the second day, although the alkalinity is not marked. Litmus is not reduced. Dextrose is fermented with the production of acid but no gas. Lactose, sucrose, and glycerin are not attacked. In lactose, sucrose, and glycerin broths the alkalinity increases until at the end of one week's incubation a pH value of 8.0 is reached. Indol is not produced.

ROD FORM

Morphology. Stained preparations from 24-hour agar cultures of this organism appear as small rods, occurring singly, and varying from 0.3 to 0.5μ in diameter and from 0.8 to 1.2μ in length. It is extremely motile, non-spore forming, and reacts negatively when stained by Gram's method. It stains readily with the ordinary bacteriological dyes.

Cultural characteristics. Moderate growth occurs on plain agar slants and in plain broth. Growth on agar slants is smooth, slightly raised, and glistening. Surface colonies are circular, finely granular, entire edge, raised, and may reach a diameter of 3 to 4 mm. Deep colonies are lens-shaped. This organism produces a yellow pigment in plain agar.

Physiology. Growth takes place readily on the surface of nutrient agar, on gelatin, and in nutrient broth. It does not form indol, reduce nitrate, liquefy gelatin, nor exert a diastatic action. Alkalinity in litmus milk is very pronounced. The entire tube of litmus milk except the surface is reduced at the

end of a 7-day incubation period. It does not attack dextrose, lactose, sucrose, nor glycerin. After 24-hours' incubation these sugar broths develop an alkaline reaction which reaches a maximum pH value of 8.2 to 8.4 after seven days of incubation.

Actinomyces were encountered in most of the washed agar plate cultures made from liquid cultures which had been exposed to the action of dyes. In a few instances these organisms were troublesome contaminants. In most cases, however, detailed colony study of the nitrite oxidizing organism and of the contaminating bacterial forms as well as isolation work could be carried on without a great deal of interference from these organisms.

SUMMARY

Numerous dyes of the phenyl-methane, xanthene, azo, and quinone-imide groups have received attention in this study. The possibilities of all of these dyes have not been exhausted, but most of them were discarded in order to permit concentration on a few of the most promising. Of this selected group of dyes rosaniline hydrochloride, a tri-phenyl methane derivative, has given the most favorable results. The two most objectionable contaminating forms can be eliminated by exposing crude cultures of the nitrite oxidizing organism to the action of this dye in 1.0 per cent concentrations for from 5 to 30 minutes. The other contaminating form can be eliminated by the use of mechanically operated sterile minute glass pipettes in fishing colonies from the surface of a solid medium.

A detailed comparison was made of the nitrite oxidizing organism and of the contaminating form which could not be eliminated by the use of rosaniline hydrochloride. This contaminating form is capable of rapid growth in nitrite medium, but only a very scanty growth on a nutrient agar. This organism is not capable of oxidizing ammonia to nitrite or of oxidizing nitrite to nitrate. It is of interest in this study because certain investigators have described an organism having similar morphological characteristics as being *Nitrobacter*. This organism stains well with cold carbol fuchsin and when stained by this dye many of the cells appeared to possess a flagellum-like attachment at one pole which in many instances was several times as long as the body of the cell. In some preparations the organisms were clustered together with the flagellum-like attachments radiating away from the center of the cluster of bacteria. In many instances the cells were unevenly stained and somewhat pointed at the ends. This organism forms a colony which can be differentiated from the colony formed by the nitrite oxidizing organism by its characteristic appearance and unevenness of edge.

In stained preparations the nitrite oxidizing organism appears singly, in pairs, and in irregular clusters. It stains evenly and varies from oval to spherical in shape. The morphology of the nitrite oxidizing organism is such that it is difficult to determine whether it should be classed as a coccus or as a rod form.

In this study it seemed desirable to refer to this organism as "the nitrite oxidizing organism" rather than to use the term *Nitrobacter* or the terminology suggested in Bergey's *Manual of Determinative Bacteriology* for those organisms oxidizing nitrite to nitrate. In the opinion of the writer the term *Nitrobacter* has been used by various workers in reference to entirely different organisms.

A detailed study was made of the morphological, physiological, and cultural characteristics of the two contaminating forms which were capable of rapid growth on ordinary culture media. These organisms are pigment-formers. One organism is a gram-negative coccus, usually occurring in pairs and forming a small circular pink colony. The other organism is a small Gram-negative, extremely motile bacillus, forming a small circular yellow colony. Both organisms were capable of rapid growth in an inorganic nitrite medium but neither was capable of oxidizing the nitrite to nitrate. By eliminating these forms from cultures of the nitrite oxidizing organisms the process of isolation is greatly simplified.

Although actinomyces were encountered in this study these organisms did not interfere to any great extent with the isolation of the nitrite oxidizing organism or with the study of the contaminating bacterial forms.

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THE VERTICAL DISTRIBUTION OF SOIL BASES AND ACIDITY IN SOME ILLINOIS SOILS[1]

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Soil profile studies are fruitful fields of investigation, and offer much promise for the future. If our understanding of soils is to be enhanced, it must come largely through greater accuracy in observations and refinement in measurements of the physical, chemical, and biological properties of the constituents of the soil profile. Much effort has been devoted to such refinements in technic in recent years.

The variations found in the profiles of soils are due principally to differences in age, rainfall, temperature, topography, and parent material. To determine how some Illinois soil profiles differ in certain chemical aspects with particular reference to reaction, base exchange, and distribution of calcium and magnesium is one of the objects of this investigation: a second objective is to study the influence of soil treatment upon these soils; and a third, to study the relation between the chemical constitution and the crop yields.

No complete review of literature is attempted in this paper, because many such reviews are already available. Among these may be mentioned the following: Way (32); Johnson (19); Gedroiz (14); Ames and Schollenberger (1); Noyes (24); Prescott (28); Fisher (12); Crowther (8); Gehring (15); Askinasi (2).

With respect to chemical studies on Illinois soils, Catherwood and DeTurk (6) made some investigations on the relation of soil treatment to exchangeable calcium and magnesium in four southern Illinois soils. They also compared soil types in regard to exchangeable and total bases, using four soils of greatly differing stages of maturity. Their findings will be referred to in connection with the results obtained in this study.

The plan of attack was to sample several soil types by 3- to 4-inch layers, using care not to include material from more than one horizon in any layer-sample. Samples were taken from fertilized as well as untreated areas. These were studied by means of the following determinations: Comber test, lime-

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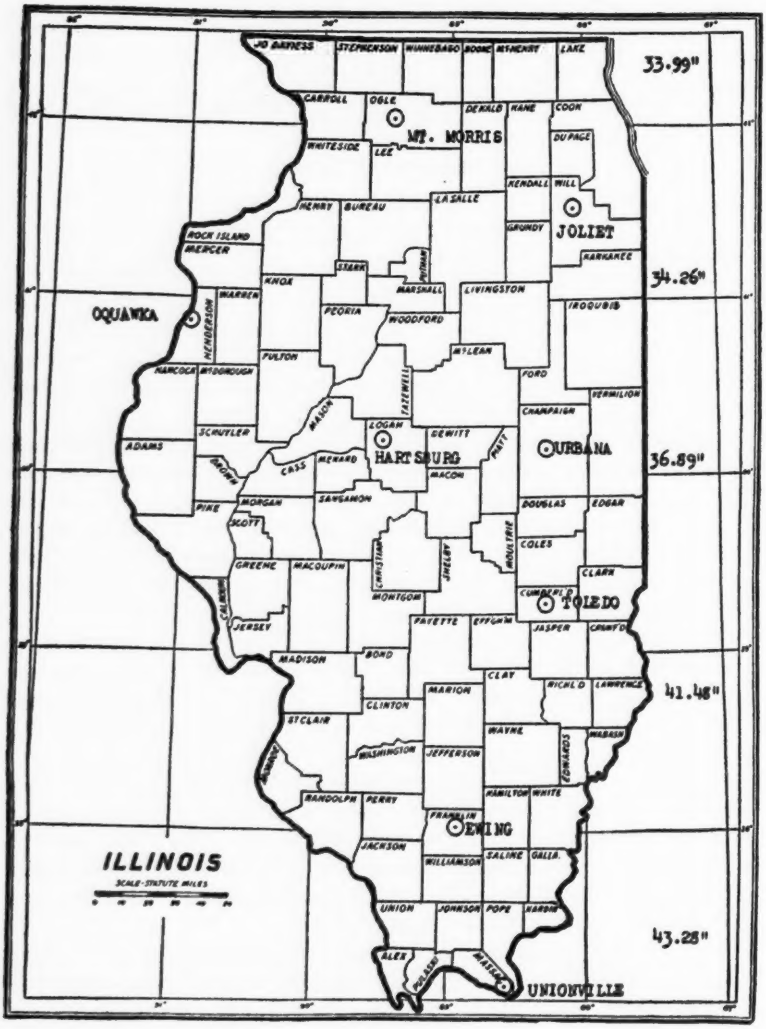

FIG. 1. MAP SHOWING LOCATION OF EXPERIMENT FIELDS FROM WHICH SAMPLES WERE TAKEN

On the right is indicated the average annual rainfall of the several latitudinal divisions of the state.

requirement by the Hutchinson-MacLennan calcium bicarbonate method and the Hopkins potassium nitrate method, hydrogen-ion concentration, buffer action, total calcium and magnesium, exchangeable calcium and magnesium, and the correlation of some of these results with crop yields.

DESCRIPTION OF SOILS, SOIL TREATMENTS, AND METHODS OF SAMPLING

The soils used in this investigation were taken from eight of the Illinois experiment fields, the basis of selection being (*a*) soil type, and (*b*) response to liming as indicated by crop yields. Samples were taken by horizons. In the case of horizons A_2 and B, if either was six inches or more thick, it was divided into two or more strata of three to four inches each. The C horizon was sampled only where it occurred within 25 to 30 inches of the surface.

Each sample was a composite of 18 borings made with the ordinary screw-type auger. Some objection may be made to the use of the auger for sampling these comparatively thin strata, and the author recognizes the limitations of this method. It would be more desirable to use a spade, making an excavation large enough to permit the observer to examine the undisturbed soil and to sample the accurately measured strata. This, of course, could not be done on the experimental plats where the study of the effect of treatment is to be continued. Moreover, a single sample taken by excavation would not necessarily represent the plat as a whole any more accurately than a composite made up of an adequate number of borings.

The location of these fields in the state is shown in figure 1. As indicated on the map, there is a gradual increase in the total annual rainfall southward (22).

All but one of the soils used in this study occur within the glaciated region. The extreme southern portion of the state in which the Unionville field is located is one of the few, small, unglaciated areas to be found in Illinois.

The profile descriptions of the soils studied and the sampling depths are as follows:

1. Ewing field (established 1910)

Gray Silt Loam On Orange-Mottled Tight Clay

HORIZONS AS SAMPLED FOR THIS STUDY	DESCRIPTION*
A_1—0 to 8″	Brownish gray silt loam
A_2—8 to 12″	Slightly mottled, brownish gray silt loam containing some yellow spots
A_3—12 to 15″	Gray silt loam heavily splotched with orange red
B_1a—15 to 18″	Highly plastic, gray clay heavily splotched with orange red
B_1b—18 to 21-22″	
C_1—21 to 24-25″	Friable, gray silty clay loam heavily splotched with yellowish brown.

Crop response to liming—R:RL = 100:352†.

* The profile description in each case has been taken from Illinois Bulletin 273 (3).

† Based on total dry weight yields for 10-year period, 1917–1926. R = residues; RL = residues + limestone.

2. Toledo field (established 1913)

Gray Silt Loam On Tight Clay

HORIZONS AS SAMPLED FOR THIS STUDY	DESCRIPTION
A_1—0 to 8″	Brownish gray silt loam
A_2a—8 to 12″	Gray silt loam
A_2b—12 to 16″	
B_1a—16 to 19–20″	Strongly mottled, yellowish brown, highly plastic clay
B_1b—19–20 to 23–28″	
C_1—23–28 to 27–33″	Gray or drabbish gray, friable silty clay loam containing many yellowish brown and black iron splotches.

This type is characterized by a great variation in the depth of the B, or compact, horizon. Crop response to liming,—R:RL = 100:223.

3. Unionville field (established 1911)

Yellow-Gray Silt Loam

HORIZONS AS SAMPLED FOR THIS STUDY	DESCRIPTION
1927 samples	
A_1—0 to 7″	Grayish yellow silt loam
A_2a—7 to 12″	Yellowish gray silt loam
A_2b—12 to 15″	
A_2c—15 to 19″	
B_1a—19 to 24″	Compact, mottled, bright yellow silty clay loam.
B_1b—24 to 28″	

1925 samples: Depths A_1—0 to 7″; A_2—7 to 20″.
Crop response to liming,—R:RL = 100:151.

4. Oquawka field (established 1915)

Dune Sand, Terrace (Plainfield sand)

"The surface is light brown between dunes, and yellowish brown on the tops of the dunes. The depth of the surface varies; it frequently is 15 inches deep between the dunes and may be entirely absent on top of the dunes. There is no horizon development below the surface, or A_1, horizon, the material consisting of incoherent yellow sand."

Sampling depths:

1. (A_1)— 0 to 8″	4.—16.5 to 21″
2. — 8 to 12″	5.—21 to 24″
3. —12 to 16.5″	6.—24 to 27″

Crop response to liming,—R:RL = 100:306.

5. Joliet field (established 1914)

Brown Silt Loam On Calcareous Drift (Clarion silt loam)

HORIZONS AS SAMPLED FOR THIS STUDY	DESCRIPTION
A_1—0 to 8″	Brown silt loam
A_2a—8 to 12″ A_2b—12 to 15″ A_2c—15 to 19.5″	Light brown or yellowish brown silt loam
B_1—19.5 to 24″	Yellowish brown, medium-compact clay, or sandy and gravelly clay
C_1—24 to 27″	Yellow, highly calcareous, sandy, and gravelly drift.

(On the Joliet field the surface soil is lighter colored, and the C horizon is reached at a shallower depth than is usual for the type).

Crop response to liming,—R:RL = 100:105.

6. Mt. Morris field (established 1910)

Light Brown Silt Loam (Tama silt loam)

HORIZONS AS SAMPLED FOR THIS STUDY	DESCRIPTION
A_1—0 to 8″	Light or yellowish brown silt loam
A_2a—8 to 12″ A_2b—12 to 16.5″	Distinctly yellowish brown, friable silt loam
B_1a—16.5 to 21″ B_1b—21 to 24″	Brownish yellow, friable, non-mottled, non-compact silt loam
C_1—24 to 27″	Very friable, slightly mottled, bright yellowish brown silt loam.

Crop response to liming,—R:RL = 100:143.

7. Urbana field (Davenport plots, established 1895)

Brown Silt Loam (Muscatine silt loam)

HORIZONS AS SAMPLED FOR THIS STUDY	DESCRIPTION
A_1—0 to 8″	Brown silt loam
A_2a—8 to 12″ A_2b—12 to 15″ A_2c—15 to 18″	Light brown or yellowish brown silt loam
B_1a—18 to 22.5″ B_1b—21.5 to 26–27″	Mottled, pale yellow, medium-compact clay loam or silty clay loam.

	crop response to liming
Series 100E	R:RL = 100:125
Series 200 W	R:RL = 100:109
Series 200 E	(Not calculated)
Series 300 W	R:RL = 100:141
Series 400 E	R:RL = 100:115

8. Hartsburg field (established 1911)

Black Clay Loam (Grundy clay loam)

HORIZONS AS SAMPLED FOR THIS STUDY	DESCRIPTION
A_1—0 to 8″	Black clay loam
A_2a—8 to 12″	Grayish or drabbish brown clay loam
A_2b—12 to 16″	
A_2c—16 to 21″	
B_1a—21 to 25″	Gray clay loam splotched with black iron concretions and yellow mottling, not very compact or plastic.
B_1b—25 to 29″	

Crop response to liming,—R:RL = 100:102.5.

The Urbana, Mt. Morris, Joliet, Ewing, and Oquawka fields were sampled between August 15 and September 15, 1926; Hartsburg and Toledo, May 1927; Unionville, April, 1925, and August, 1927.

Soil treatments

For those portions of the experimental fields used in this study the standard plan of soil treatments is as follows:

Plot	6 R	residues
	7 RL	residues and limestone
	8 RLP	residues, limestone and phosphate
	9 RLPK	residues, limestone, phosphate and potash
	10 0	check

This is true for each field except Urbana, where the arrangement is:

Plot	1 0	check	
	2 R	residues	
	4 RL	residues and limestone	
	6 RLP	residues, limestone,	bone phosphate E half rock phosphate W half
	8 RLPK	residues, limestone, potash	bone phosphate E half rock phosphate W half

The total amount of limestone that has been applied to the soils varies from 13,000 to 20,000 pounds per acre.

METHODS OF ANALYSIS

Comber potassium thiocyanate test

Approximately 2 gm. of 10-mesh soil (measured) and 5 cc. of alcoholic potassium thiocyanate (40 gm. thiocyanate in a liter of 95 per cent alcohol) were vigorously shaken together in a test tube for a few seconds, and the readings taken after about 15 minutes. It was found that it is very essential to avoid grinding the samples for this test.

Hutchinson-MacLennan lime requirement method

Ten grams of air-dry, 10-mesh soil were shaken one hour with 100 cc. of approximately 0.017 *N* calcium bicarbonate, precautions being taken to keep an abundance of carbon dioxide present in order to prevent possible precipitation of calcium carbonate. The suspension was filtered and a 50-cc. aliquot titrated against 0.1 *N* acid.

Hopkins lime-requirement test

A 100-gm. sample of 10-mesh soil was shaken for one hour with 250 cc. of normal potassium nitrate solution. After settling, a 125-cc. portion of the supernatant liquid was drawn off and titrated against 0.1 *N* sodium hydroxide. The titration figure was multiplied by the factor 2.5 (18), and the result presented as grams of calcium carbonate required by 100 gm. of soil.

Hydrogen-ion concentration

The hydrogen-ion concentration, expressed as pH, was determined electrometrically, the hydrogen electrode (4) being used. One hundred mesh soil was used throughout, with the exception of the Oquawka sand, which was used in the unground condition. All determinations were run in duplicate.

Indications from observations of the author and others are that the grinding of a soil increases the pH value slightly in the silt loams and clay loams, and considerably in the case of sands.

Buffer action

Ten-gram samples of 20-mesh soil were shaken one hour with varying amounts of 0.038 *N* calcium hydroxide and enough water to make 20 cc. of liquid. At the end of that period the pH was determined, using the quinhydrone electrode. This method gave very satisfactory results with all except the Unionville soil. In the latter case, the results were nearly 2 pH higher than the figure obtained by the hydrogen electrode. Repeated trials with other samples from the same field gave the same results. This brings out the weak point in the quinhydrone method—occasionally it will give erratic results. Final data on this soil were secured by using the hydrogen electrode. Buffer action was determined only in the A_1 horizon of the unlimed soils.

Total calcium and magnesium

The estimation of calcium and magnesium was made in accordance with standard methods, using the following general procedure: Fusion of the 100-mesh soil with Na_2O_2; double precipitation of R_2O_3; single precipitation of CaC_2O_4, which was titrated with $KMnO_4$; and double precipitation of the $MgNH_4PO_4$ which was ignited to $Mg_2P_2O_7$. All samples were analyzed in duplicate.

Exchangeable calcium and magnesium

Because of the large number of samples to be analyzed in the limited time available, it was necessary to choose a method that would not be too cumbersome and time-consuming. Furthermore, as some of the samples contained carbonates, the solubility of these carbonates in the displacing solution had to be taken into consideration. Normal solutions of potassium chloride and ammonium acetate, and a 0.05 N solution of hydrochloric acid were tried as displacing agents, with more or less success. Although the solutions varied in their ability to dissolve carbonates, all of them took into solution large enough amounts—especially from the Joliet subsoil which was particularly calcareous—to give high results for calcium. In order to correct for the amount of calcium carbonate dissolved, it was necessary to determine the carbonates in the soil before and after leaching.

Procedure adopted for determining exchangeable calcium and magnesium. Twenty-five grams of air-dried soil were placed in a beaker, and about 150 cc. of normal ammonium acetate added. This was stirred several times, and after four or five minutes the mixture was transferred quantitatively to a 10-cm. Buchner funnel, using a good grade of ordinary rapid filter paper such as S & S 589 white ribbon, and using light suction. A total of 750 cc. was passed through the soil in small portions, the filtrate evaporated to dryness, the acetate driven off and the organic matter partially removed by ignition on a silica plate over a Bunsen burner. The remaining organic matter was further treated with aqua regia, followed by dehydration.

The R_2O_3 compounds were removed with ammonium persulfate and ammonium hydroxide, and the calcium and magnesium determined, using double precipitation in each case. All extractions were made in duplicate, with single determinations on each extract.

In the first few sets, the R_2O_3 precipitate was ignited, weighed, and recorded. The amounts obtained, however, were consistently low.

Where carbonates were present in measurable quantities they were determined by the use of HCl in the Parr apparatus, before and after leaching the soil with ammonium acetate. The carbonate calcium dissolved by the salt solution was subtracted from the total amount of calcium in the filtrate.

This method of correcting for carbonates dissolved is not without fault, because of the depressing influence of calcium in solution upon calcium replacement. Where both calcium carbonate and magnesium carbonate are present the method cannot be used with any degree of accuracy, since there is no satisfactory way to distinguish between the quantity of the two kinds of carbonate in the soil, or between the amount of each dissolved and the amount of calcium and magnesium that is being replaced. MacIntire (21) presented data to show the non-existence of magnesium carbonate in humid soils.

In this investigation it is assumed that, except in the subsoil of the Joliet fields, no magnesium carbonate was present in these soils.

In the case of the Joliet subsoil samples the amount of carbonate found by analysis exceeded (when calculated as calcium) the amount of total calcium in the soil. Therefore, we are led to believe that magnesium carbonate does exist as such in this soil. It should be noted also that the total magnesium content of this stratum is very high. The data for exchangeable calcium in

the Joliet subsoil were obtained by assuming the amount of exchangeable calcium of the C_1 horizon to be commensurate with that found in the overlying B_1, which contained only a small amount of carbonate.

The experience of the writer in respect to the carbonate question bears out the accuracy of Gedroiz's observation: "Quite a considerable number of soils that we have investigated containing either $CaCO_3$ alone, or $CaCO_3$ and $MgCO_3$ lead us to the conclusion that, without further development of the methods for these cases, it is very risky to base any conclusions on the data for zeolitic Ca and Mg obtained by the method described here"[3] (13).

In analyzing the soils for inorganic carbonates with the Parr apparatus, using 1:1 hydrochloric acid as the decomposing agent, it was observed that carbon dioxide was obtained from some of the most acid samples. This occurred particularly in the upper horizons of the Unionville and Mt. Morris soils. Both phosphoric and sulfuric acids were tried with identical results. Acetic acid, however, liberated no carbon dioxide from the acid soils, and in the case of limed soils gave slightly lower results than did hydrochloric acid.

It was found that acetic acid was just as effective as hydrochloric acid in decomposing calcium carbonate. Apparently hydrochloric acid reacts on the soil in a way different from acetic acid. Whether or not it is an organic decomposition is not known. Hissink (17) was confronted by the same problem, having obtained carbon dioxide from a soil with a pH value of 4.58. Since his soils were very rich in organic matter he suggests that possibly a small amount of carbon dioxide is formed by oxidation of the carbon. However, in the case of the Illinois soils this liberation of carbon dioxide is not correlated with the amount of organic matter in the soil. Neither is it the result of boiling the suspension containing hydrochloric acid, since the same amount of carbon dioxide was obtained in the cold under reduced pressure.

PRESENTATION OF DATA

The data obtained in this investigation will be presented under the following headings: Comparison of Soil Types; Comparison of Soil Treatments; Correlation of Crop Yields with Laboratory Results.

Comparison of soil types

The untreated plots only are considered in the comparison of soil types. The results are shown in figures 3, 4, and 5. For the most part the data have been taken from plot 10 on each field excepting Urbana, where no. 1 is the untreated plot. In a few instances not all of the data were available for plot 10, and in these cases the information was taken from the residue plot, no. 6, on which, as on plot 10, no minerals have been applied.

[3] Gedroiz refers to the determination of carbonates before and after leaching, and subtracting the calcium equivalent of the amount dissolved from the amount of calcium found in the filtrate.

Hydrogen-ion concentration.—The hydrogen-ion concentration decreases with the increasing depth in every case, although the decrease is very slight in the Mt. Morris and the Oquawka soils. These two soils exhibit considerable uniformity throughout their profiles in comparison with the other soils studied in this work. The subsoil on the Joliet field is markedly calcareous, which fact accounts for the great decrease in hydrogen-ion concentration.

It is interesting to note that the H-ion concentration of the lower strata of the Toledo soil,—Gray Silt Loam on Tight Clay,—is considerably less than that of the corresponding layers in the Ewing soil,—Gray Silt Loam on Orange-Mottled Tight Clay.

The low H-ion values in the lower levels of both of these soils indicate a "slick spot" conditions, since all soils examined in this region except "slick spots" have definitely higher H-ion concentrations in the B and C horizons.[4] The lower H-ion concentration in the Toledo soil indicates a more pronounced development of "slick spot" formation. Although the amounts of replaceable calcium and "magnesium" are apparently sufficient to account for the reaction as found, these "slick spots" are characterized by the presence of replaceable sodium as well. Qualitative tests on these samples showed fairly large amounts of sodium.

Comber test.—In a very general way, the intensity of color of the Comber test follows the pH values. This shows that the Comber test is a remarkably good indicator of the H-ion concentration of the soil, and it can usually be relied upon qualitatively.

Buffer action.—Although the H-ion concentration is the most reliable measure of the acidity of the soil, it does not indicate the amount of lime that is necessary to bring an acid soil to the neutral point. This latter value may be determined with considerable accuracy by an electrometric titration of the soil with a base, as has been described under "Methods of Analysis."

The results—obtained only on the surface samples of the unlimed soils—are to be shown in figure 2. As indicated by the steepness of the curves, the Oquawka sand possesses the least buffer power, and the Unionville, Ewing, and Toledo soils come next with a buffer capacity considerably greater than the sand but less than the remaining northern soils.

A method which gives results that are directly comparable is to estimate from figure 2 the amount of calcium hydroxide required to change the pH from one value to another. By eliminating Hartsburg and Oquawka, and extending the Urbana curve to 7.0, it is possible to compare directly six of the soils from pH 5.5 to 7.0. The data are given in table 1.

In order to include Hartsburg and Oquawka, we can use the values 6.3 and 7.5, with the subjoined data (table 2).

We see from table 1 that the Urbana soil is the most highly buffered of the

[4] Norton, E. A., and R. H. Bray. The Reactionary Soil Profile. To appear in *Jour. Amer. Soc. Agron.*

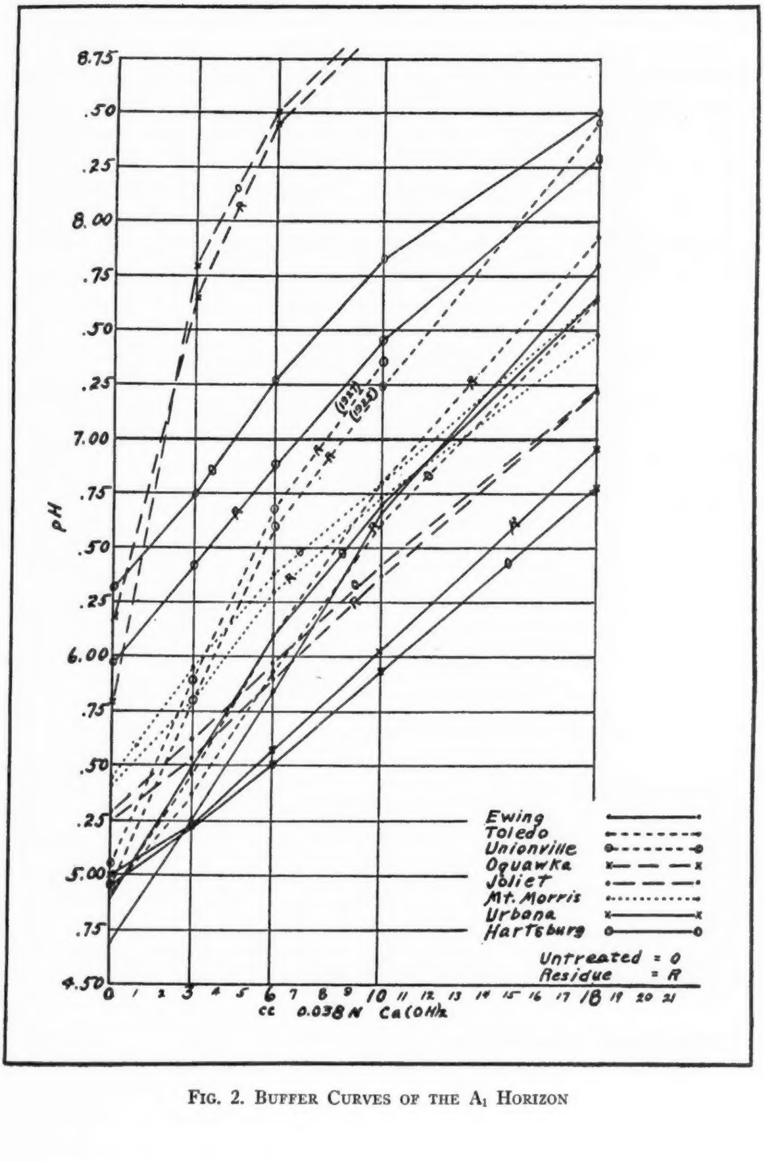

FIG. 2. BUFFER CURVES OF THE A_1 HORIZON

six, whereas Unionville has the least buffering power. In the case of table 2 Oquawka is least buffered, and Hartsburg is about the same as Toledo.

Lime-requirement.—The Hutchinson-MacLennan and the Hopkins lime-requirement methods paralleled the pH values in some cases, and in other cases they ran counter to those values.

Smith (29) working with Scottish soils, reports fair agreement between the results obtained by the Hutchinson-MacLennan method and the pH values. Crowther and Martin (11) say that the results obtained by the Hutchinson-MacLennan method are always lower than the equivalent amount of calcium hydroxide required by the electrometric titration method. They state further that the Hutchinson-MacLennan method is no indicator of the intensity of

TABLE 1

Amount of 0.038 N $Ca(OH)_2$ required to change the pH from 5.5 to 7.0

SOIL	UNTREATED	RESIDUE	SOIL	UNTREATED	RESIDUE
	cc.	*cc.*		*cc.*	*cc.*
Ewing	9.6	8.1	Joliet	13.7	13.3
Toledo	9.3	8.3	Mt. Morris	11.6	12.2
Unionville	6.3	6.5	Urbana	14.1	13.0

TABLE 2

Amount of 0.038 N $Ca(OH)_2$ required to change the pH from 6.3 to 7.5

SOIL	UNTREATED	RESIDUE	SOIL	UNTREATED	RESIDUE
	cc.	*cc.*		*cc.*	*cc.*
Ewing	9.3	7.7	Mt. Morris	11.2	12.1
Toldeo	8.7	7.8	Joliet	11.6	11.5
Unionville	6.2	6.8	Hartsburg	7.8	8.2
Oquawka	1.8	2.4			

soil acidity but merely gives an indication of the amount of lime needed to reduce considerably the acidity of the soil.

On the other hand, Brioux and Pien (5) state that the Hutchinson-MacLennan method compares very favorably with electrometric titration to a pH of 8.0. Ogg and Dow (26) report close agreement between the pH values and the Hutchinson-MacLennan data in soils of similar colloidal content.

The data obtained in this study agree with those of the last two references. The figure which was obtained by the Hutchinson-MacLennan method representing the amount of calcium carbonate required by the soil was divided by the calcium carbonate equivalent of 1 cc. of standard calcium hydroxide used in determining the buffer action, giving the cubic centimeters of hydroxide that would be required to equal the first mentioned figure. By interpolation on the graphs then, it was found that the Hutchinson-MacLennan method compared

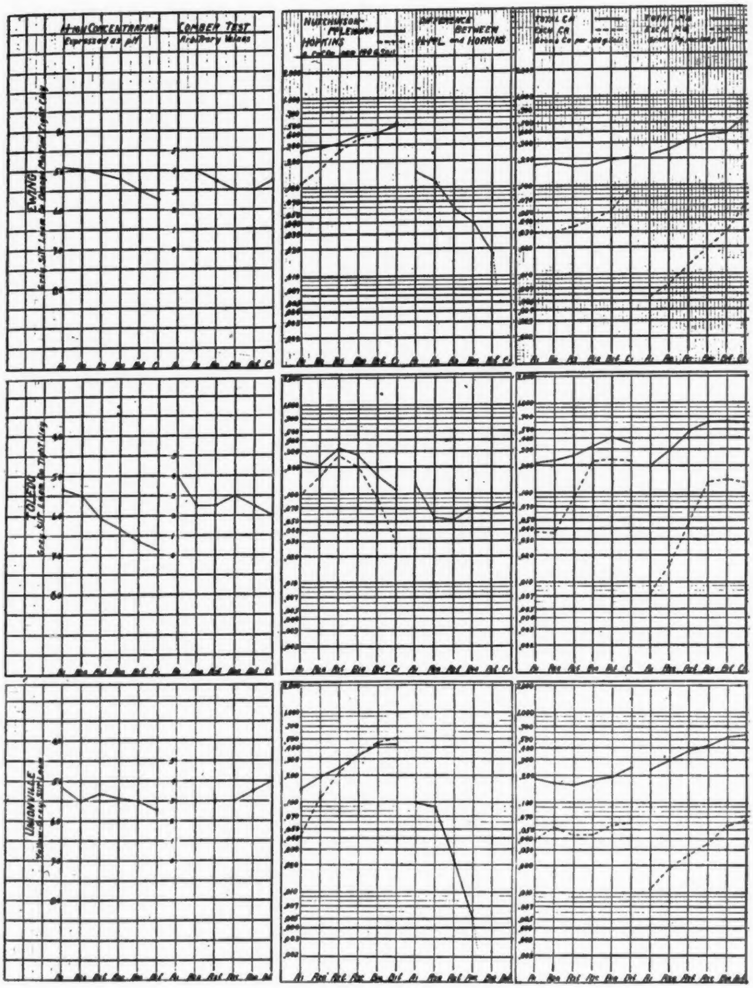

FIG. 3. GRAPHIC PRESENTATION OF DATA FROM UNTREATED PLOTS OF THE EWING, TOLEDO AND UNIONVILLE FIELDS

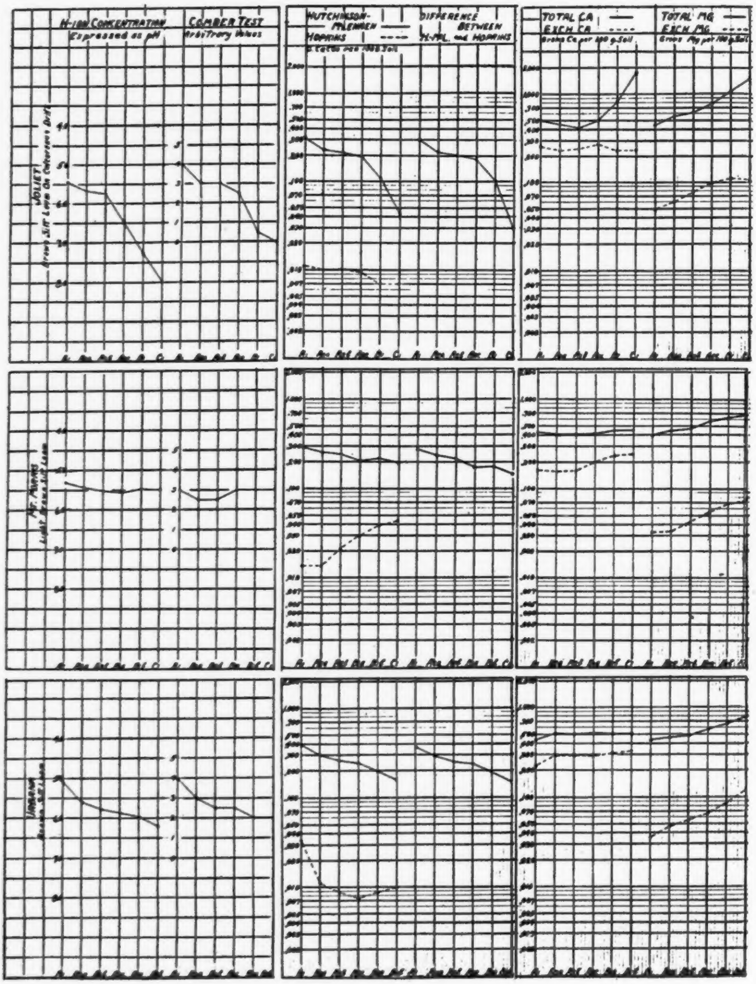

FIG. 4. GRAPHIC PRESENTATION OF DATA FROM UNTREATED PLOTS OF THE JOLIET, MT. MORRIS, AND URBANA FIELDS

quite favorably with the electrometric titration to a pH of approximately 7.0. Taking the Ewing soil for example:

$$\frac{\text{H.} - \text{MacL. lime req. per 1,000,000}}{CaCO_3 \text{ equiv. of 1 cc. of 0.038 } N \text{ } Ca(OH)_2} = \frac{2590}{190} = 13.6 \text{ cc.}$$

A line extended from 13.6 cc. on the graph in figure 2 intercepts the Ewing curve at pH 7.12. These data are shown in table 3.

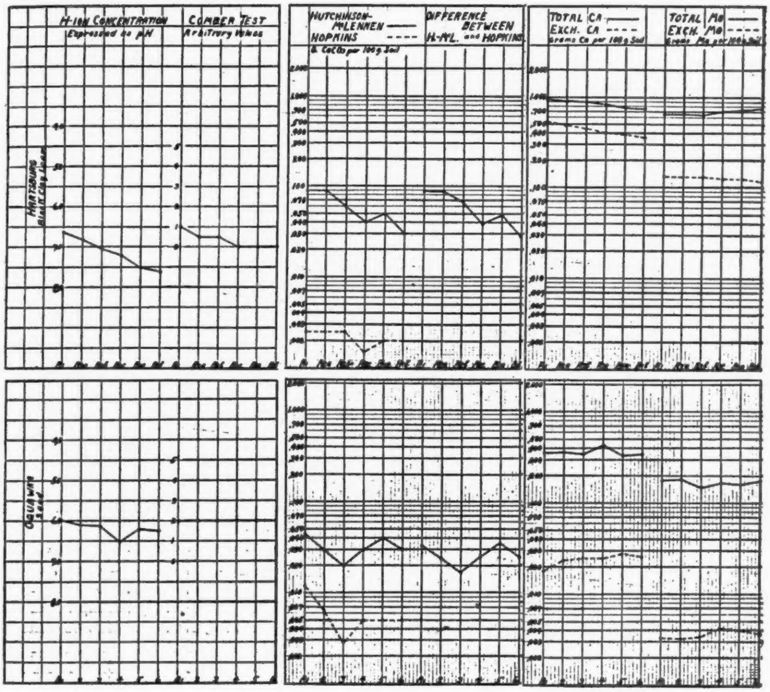

FIG. 5. GRAPHIC PRESENTATION OF DATA FROM UNTREATED PLOTS OF THE HARTSBURG AND OQUAWKA FIELDS

Figures 3, 4, and 5 show that only in the soils of Southern Illinois—Ewing, Toledo, and Unionville,—do the lime-requirement values run counter to the pH; and in fact it is only in those soils that the lime-requirement increases with increase in depth. This latter statement is only partly true for the Toledo soil, since its points of high acidity as measured by both lime-requirement methods occur in layers A_2b and B_1a, or in other words at the juncture of the A and B horizons. There is no sign of such a "hump" in the curve representing pH. The curve shown under the caption, "Difference Between H.-MacL. and Hopkins" is pretty largely the reverse of the lime-requirement curves.

At this point it is well to consider briefly the question of soil acidity as measured by different methods. Askinasi (2) classifies soil acidity as follows:

A. Active acidity,—in which a water extract of a soil contains free H ions; the acidity which is brought about by free acids or their acid salts.

B. Passive acidity,—in which the H ion is in the inactive, absorbed state. It is detectable by treatment of the soil with a salt solution.

(1) Unsaturation (exchange acidity),—in which the H ion is displaced by neutral salts.

(2) Hydrolytic acidity,—in which the H ion is displaced only by free alkalies or alkali-reacting salts.

TABLE 3

pH values obtained by interpolation. A comparison of Hutchinson-MacLennan lime requirement method with electrometric titration

FIELD	TREATMENT	LIME REQUIREMENT BY H. MacL., (cc. $Ca(OH)_2$)	pH
Ewing	O	13.6	7.12
	R	12.8	7.06
Toledo	O	12.1	6.86
	R	11.6	7.02
Unionville	R	7.4	6.93
	R	8.9	7.06
Oquawka	O	2.4	7.37
	R	1.8	7.00
Joliet	O	16.5	7.08
	R	16.5	7.05
Mt. Morris	O	15.2	7.34
	R	16.5	7.33
Urbana	O	21.0	7.09
	R	20.0	7.18
Hartsburg	O	4.7	7.04
	R	7.4	7.08

Under B (1), Askinasi includes the methods of Gedroiz, Daikuhara, Hopkins, and others. All unsaturated soils he considers as belonging to one of three groups: 1. Soils which contain a large amount of active aluminum; 2. Soils which contain only a small amount of active aluminum; 3. Soils which contain no active aluminum. In the treatment of the soils of the first group with a neutral salt, the mineral acid resulting from the exchange process, e.g. Soil H + KCl $\rightleftarrows$ Soil K + HCl, is "neutralized" almost entirely by the Al. With the soils of the second group, only a part of the H ion is "neutralized" by the Al.

In this case, both H ion and Al ion are present in the filtrate. When the soils of the third group containing no aluminum are treated with neutral salts only the free mineral acid is found in the filtrate.

Hydrolytic acidity is characterized by the ability of the soil to neutralize the product of the hydrolysis of a salt that is composed of a strong base and a weak acid. For example, sodium acetate hydrolyzes as follows:

$$CH_3COONa + H_2O \rightleftarrows CH_3COOH + NaOH$$

In an acid soil the Na ion replaces the adsorbed H ion of the soil and water is formed. The equilibrium of the above reaction is not established until all of the soil H ion has been displaced by the Na ion.

Therefore, the treatment of an acid soil with the salt of a strong base and a weak acid measures both exchangeable and hydrolytic acidity. For the ascertainment of the latter then, the results of a neutral salt extraction must be subtracted from the results secured by treatment with an alkali or an alkali-reacting salt such as sodium acetate.

In the Hutchinson-MacLennan method calcium bicarbonate is used rather than sodium acetate. The difference between the results obtained by the Hutchinson-MacLennan and the Hopkins methods, shown in figures 3, 4, and 5, therefore, represent essentially the hydrolytic acidity as defined by Askinasi (2) and others (7), (20).

It is seen that only in the soils from Ewing, Toledo, and Unionville does the hydrolytic acidity differ to any consequential degree from the total acidity as indicated by the calcium bicarbonate method. For only in these three soils does the exchange acidity approach closely or exceed the total acidity.[5] Thus it can be said that these three soils are characterized by a comparatively high total acidity, and of this acidity a very large proportion is described as exchangeable, leaving a very small part as hydrolytic acidity.

Total calcium.—The relationships of total calcium to pH are not at all uniform. On the Mt. Morris field the curve for total calcium follows very closely that of the pH values; on the Hartsburg field there is less correlation between the two; whereas on the other fields it varies more widely. These results are similar to those reported by Némac and Gracanin (23).

Total magnesium.—As was the case with total calcium the correlation between total magnesium and pH is quite variable. The lower horizons of all types, excepting the Oquawka sand, contain more magnesium than do the surface layers. MacIntire (21) states that magnesium does not usually exist in humid soils as the carbonate, but is converted to silicates, whereas calcium is found partly as the carbonate and partly as the silicate, both of which are more readily leached than is magnesium silicate.

[5] Naturally it is impossible for the exchangeable acidity to exceed the total acidity. Where such appears to be the case it merely indicates that the absolute values of the two methods cannot be compared directly. When compared relatively, however, the figures are significant.

In nearly every instance in the surface horizon, the percentage of total magnesium is approximately the same as the total calcium.

Exchangeable calcium and magnesium.—The exchangeable calcium content follows the total calcium for the various depths within each soil type, but in most instances the percentage increase or decrease in the exchangeable is greater than the corresponding change in the total. This statement holds true also for exchangeable magnesium in relation to total magnesium. The exchangeable calcium in the horizons B_1 and C_1 of the Joliet soil does not follow the total calcium content, as far as could be determined. As stated previously, the high content of carbonates interferes seriously with the accurate estimation of the replaceable bases of a soil and in the case of C_1 horizon of the Joliet soil this value was taken to be the same as that for B_1.

Page and Williams (27) reported that the amount of exchangeable calcium in a number of the soils which they studied was closely correlated with the pH values. With some Scottish soils Smith (29) found no real correlation in this respect except in soils of similar character.

TABLE 4

Exchangeable calcium and magnesium in surface (A_1) horizon expressed as percent of total calcium and magnesium respectively

FIELD	Ca	Mg	FIELD	Ca	Mg
Ewing	17.1	2.5	Joliet	51.0	11.1
Toledo	17.4	3.7	Mt. Morris	39.2	8.2
Unionville	21.3	4.7	Urbana	51.0	8.0
Oquawka	5.4	1.7	Hartsburg	55.8	20.4

A very interesting point to observe is the difference in the proportion of total base which is in the exchangeable form in the various soils. This can be seen in the graphs in figures 3, 4, and 5. The data for the surface samples only are here presented.

It is readily seen that the northern soils fall into one group and the southern soils into a second group in this regard. The one exception is Oquawka sand which because of its low colloid content, falls into the southern rather than the northern group.

Catherwood and DeTurk (6) studying four soil types of Illinois—Light Gray Silt Loam On Tight Clay, Brown Gray Silt Loam On Tight Clay, Brown Silt Loam, and Black Clay Loam—found the percentage of total calcium present in exchangeable form to be least in the Light Gray Silt Loam, and increasing in the several types in the order given.

It is interesting to note that the pH of the A_1 horizon of all but the Hartzburg and Oquawka soils lies between 4.97 to 5.5. The total calcium of this layer of the three southern fields is found in the vicinity of 0.2 per cent, but in the three northern fields it lies between 0.4 and 0.5 per cent. Again, with the

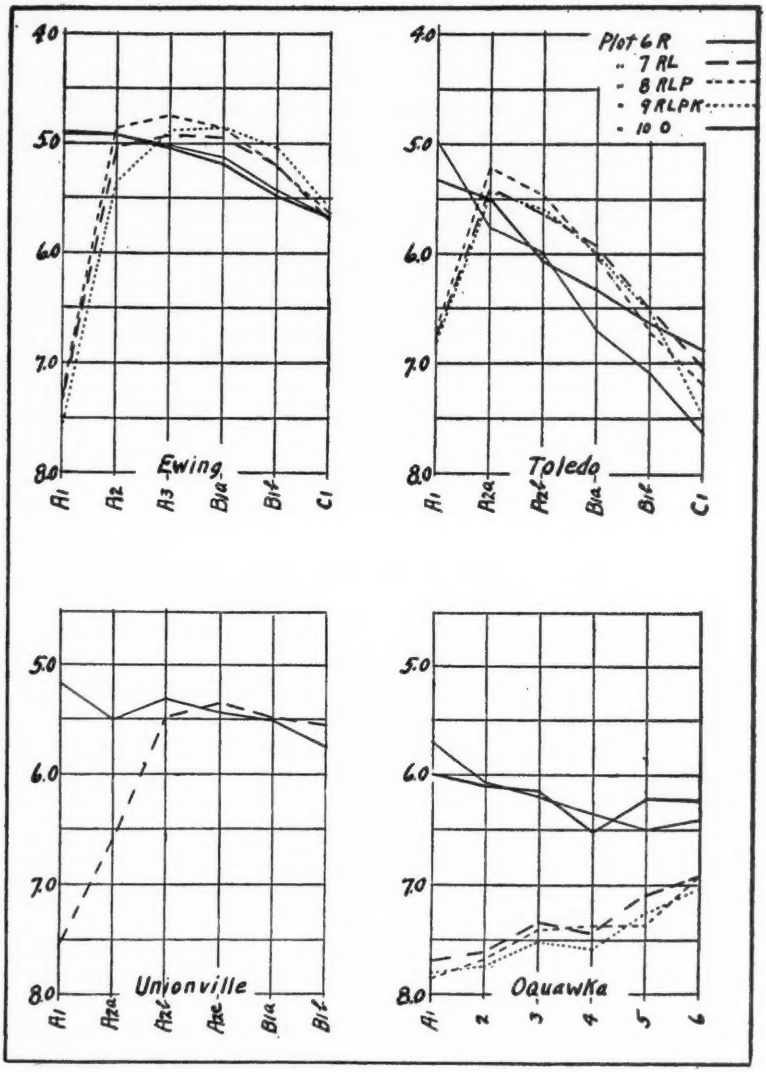

FIG. 6. EFFECT OF TREATMENTS ON H-ION CONCENTRATION (EXPRESSED AS pH) OF SOILS FROM FOUR FIELDS

same pH, the southern soils have an exchangeable calcium content within the limits of 0.026 and 0.038 per cent, whereas that of the northern soils is much higher, namely between 0.16 and 0.27 per cent.

In both the untreated and the residue plots there is no carbonate carbon in the upper layers of the Joliet soil nor in the entire profile of the other soils.

The high carbonate content of the subsoil on the Joliet field has little influence on the surface horizon of that soil, for one may observe the similarity in the chemical data of the A_1 horizons of the Joliet and Mt. Morris soils, although the Mt. Morris soil lacks carbonates and is quite uniformly acid in all horizons.

Comparison of soil treatments

The previous section dealt with a study of the untreated plots of the various soil types. The effects of soil treatments upon the soils in question as registered in the chemical studies will now be considered. The data for the treated as well as the untreated plots are shown in figures 6 to 17. The symbols used in these figures have the following significance:

O = No treatment
R = Non-legume crop residues, including wheat and oats straw, and corn stalks. Straw has been discontinued during the last four to five years. Legume catch crops are also included.
L = Limestone
P = Rock phosphate except on the east half of Urbana plots (marked *E*) where it represents steamed bone meal.
K = Kainit

Limestone has reduced the H-ion concentration of the surface layer in every case (figs. 6, 7, 8). Its effect upon the H-ion concentration has extended only through the A_1 horizon of the Ewing and Toledo soils; to the A_2b layer at Unionville, Joliet and Mt. Morris; to the A_2c layer at Urbana; to the B_1a layer at Hartsburg, and through all six layers at Oquawka. The data show somewhat lower pH values in the limed plots for the intermediary horizons of the Ewing and Toledo soils, but it would hardly be safe to ascribe these changes to the limestone treatments, especially when none of the data for lime-requirement or for total or exchangeable calcium and magnesium show corresponding changes in these horizons.

Crowther (9) studying the soils on the Rothamsted and Woburn fields found that on a medium acid soil which had received 3.8 tons of lime per acre for a period of 17 years the lime was responsible for an increase of 1.3 in pH, which from titration curves of one of the plots indicated a lime-requirement of two tons for the surface nine inches. He states that as a result of soil treatment the subsoil underwent changes similar to, but lesser in degree than those of the surface soil.

In his succeeding article (10), Crowther concludes in part,

The reactions of the unmanured and the limed and unlimed portions of the sulfate of ammonia plots on Rothamsted Park Grass and Woburn barley fields change steadily with

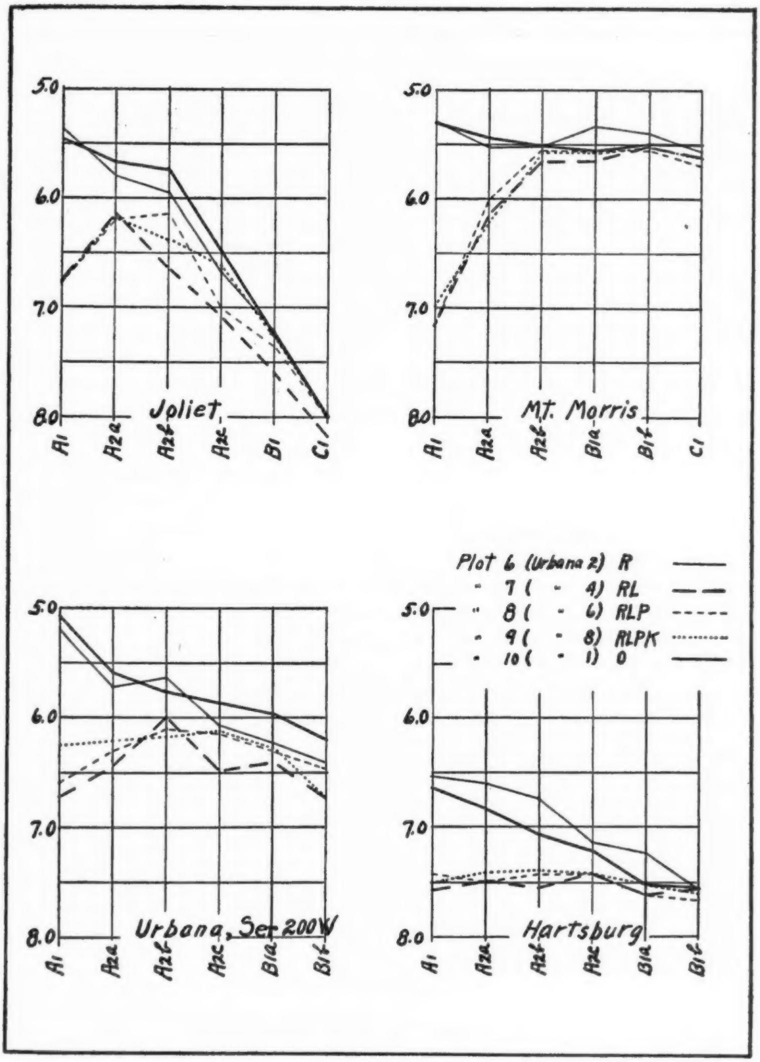

Fig. 7. Effect of Treatments on H-ion Concentration (Expressed as pH) of Soils from Four Fields

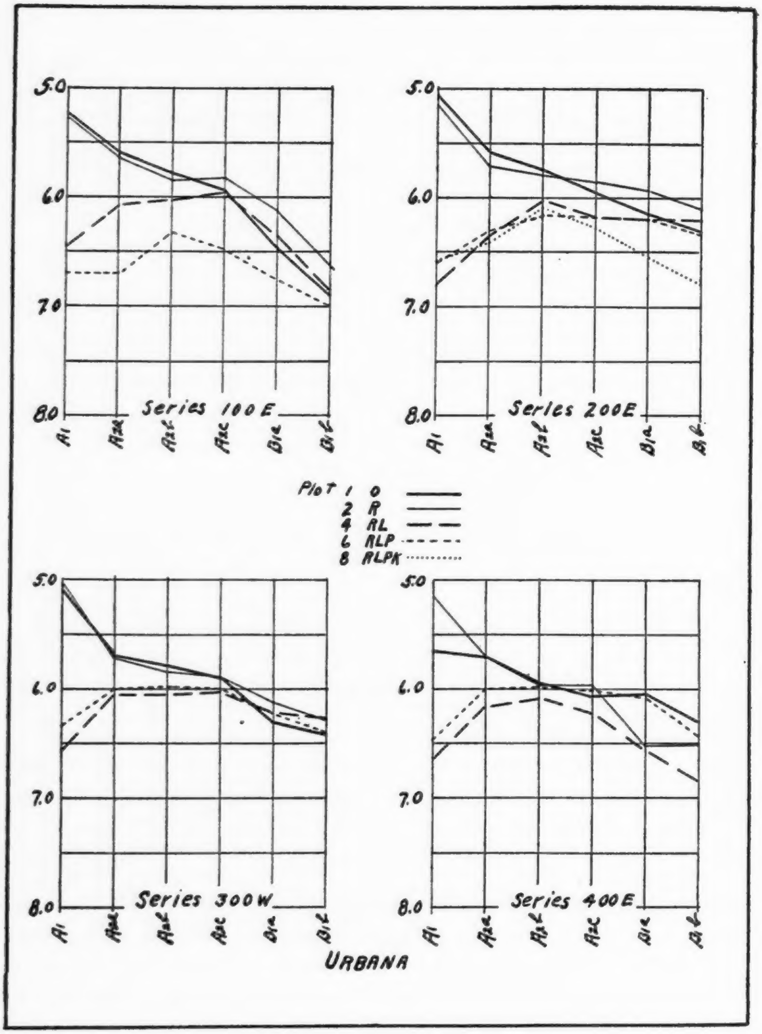

FIG. 8. EFFECT OF TREATMENTS ON H-ION CONCENTRATION (EXPRESSED AS pH)

For series 200W see figure 7

increasing depth, and at 36 inches still show the same relations as in the surface soil. The difference in pH values between the limed and unlimed portions of the Rothamsted soil is substantially constant at all depths down to 36 inches. The reaction of the subsoil plays an important part in determining the effect of liming.

The data which have been presented indicate that the response to liming of the Illinois soils studied differs markedly from the response observed by Crowther in the English soils.

The pH data for Urbana Series 200W are given in figure 7, and those for the remaining four series sampled at Urbana are to be found in figure 8. Comparison of the five series with one another shows similarity in the curves, and at the same time shows the soil variations. A striking similarity is to be observed between the variations in this soil as indicated by the H-ion concentration (fig. 8) and the variations registered by the Hutchinson-MacLennan lime-requirement test (fig. 12).

The influence of limestone is detected by this latter test to varying depths in the several soils. This influence, however, does not in all cases coincide with the pH data in this respect.

With the possible exception of the soil on the Ewing field, no effects of liming can be observed by the Hopkins test below A_1.

Ames and Schollenberger (1) estimated the lime-requirement of a limed Wooster silt loam by 4-inch layers to a depth of 24 inches by the Hopkins, Veitch, and Vacuum methods. They found the effect of lime reaching to 24 inches or more. From results obtained by the Hopkins tests, Stewart (31) found that on Gray Silt Loam On Tight Clay of southern Illinois, limestone applied to the surface penetrated slowly into the second stratum (7 to 20 inches) but not at all into the lower sampling stratum (20 to 40 inches). His second stratum (7 to 20 inches) was not subdivided in sampling.

There is no conclusive evidence in the data here presented that soil treatments *in addition to liming* have had any influence upon the lime-requirement of these soils, or upon the amount of replaceable calcium and magnesium. In other words, the total effect of soil variation and of errors in sampling and in analysis is greater than any differences due to soil treatments other than liming.

In his work on Illinois soils, Catherwood[6] was unable to detect any increase in exchangeable calcium in plots which had been treated with bone phosphate alone or in conjunction with potash; but when limestone was included in the treatment, bone phosphate showed a marked increase in exchangeable calcium over the limestone-without-phosphate plot.

In this connection can be mentioned the work of Smith (30) in which he sampled several soils before and after an interval of 12 months. Some differences were obtained in the exchangeable calcium content which he attributed to field error rather than to cropping.

The fact that, in this work on Illinois soils, liming seems to have influenced

[6] Catherwood, M. P. Thesis, University of Illinois, 1927.

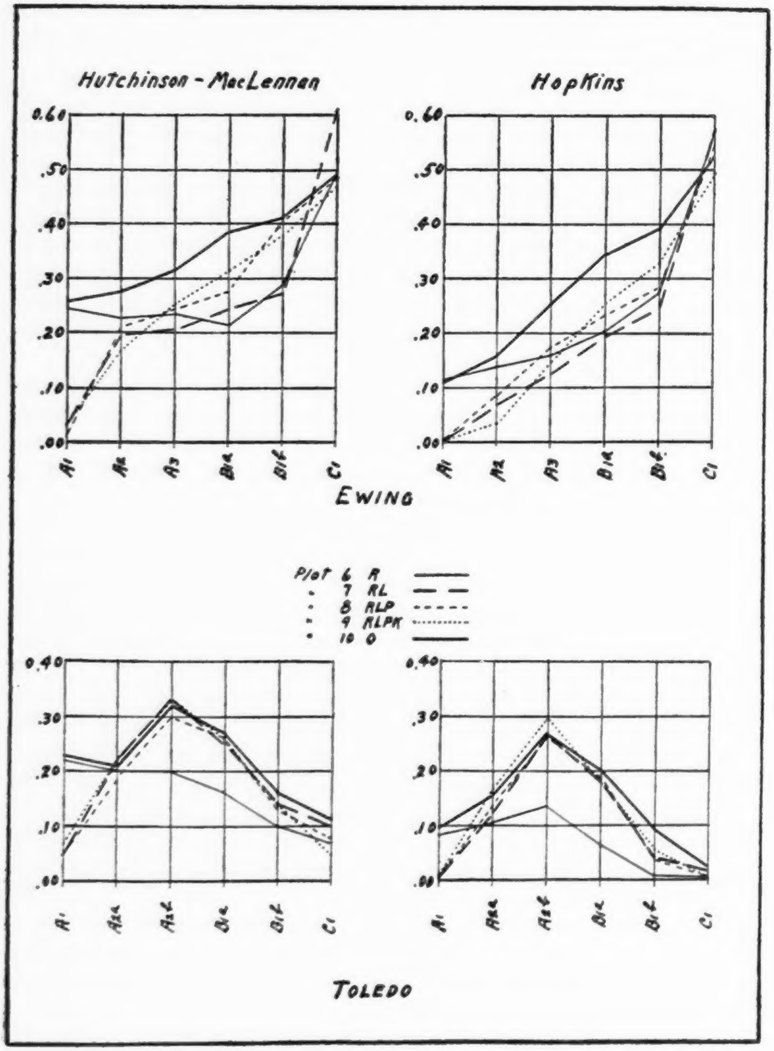

FIG. 9. EFFECT OF TREATMENTS UPON THE SOIL LIME REQUIREMENT AS MEASURED BY THE HUTCHINSON-MACLENNAN AND THE HOPKINS METHODS IN GRAMS $CaCO_3$ PER 100 GM. SOIL

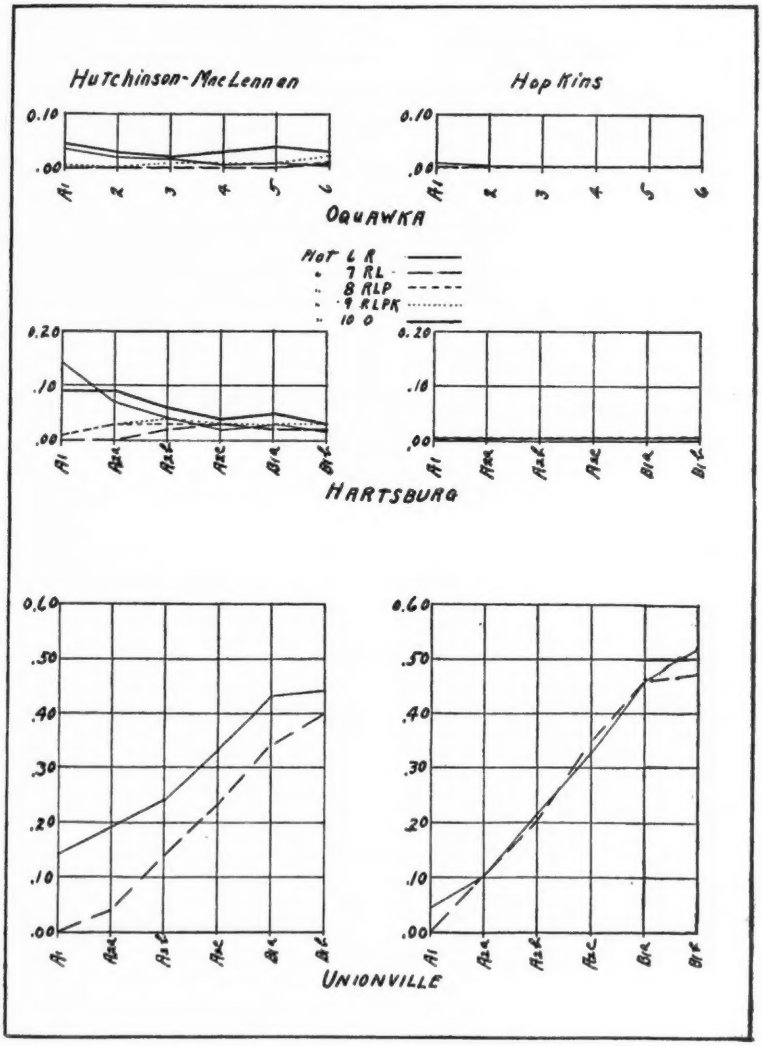

FIG. 10. EFFECT OF TREATMENTS UPON THE SOIL LIME REQUIREMENT AS MEASURED BY THE HUTCHINGON-MACLENNAN AND THE HOPKINS METHODS, IN GRAMS $CaCO_3$ PER 100 GM. SOIL

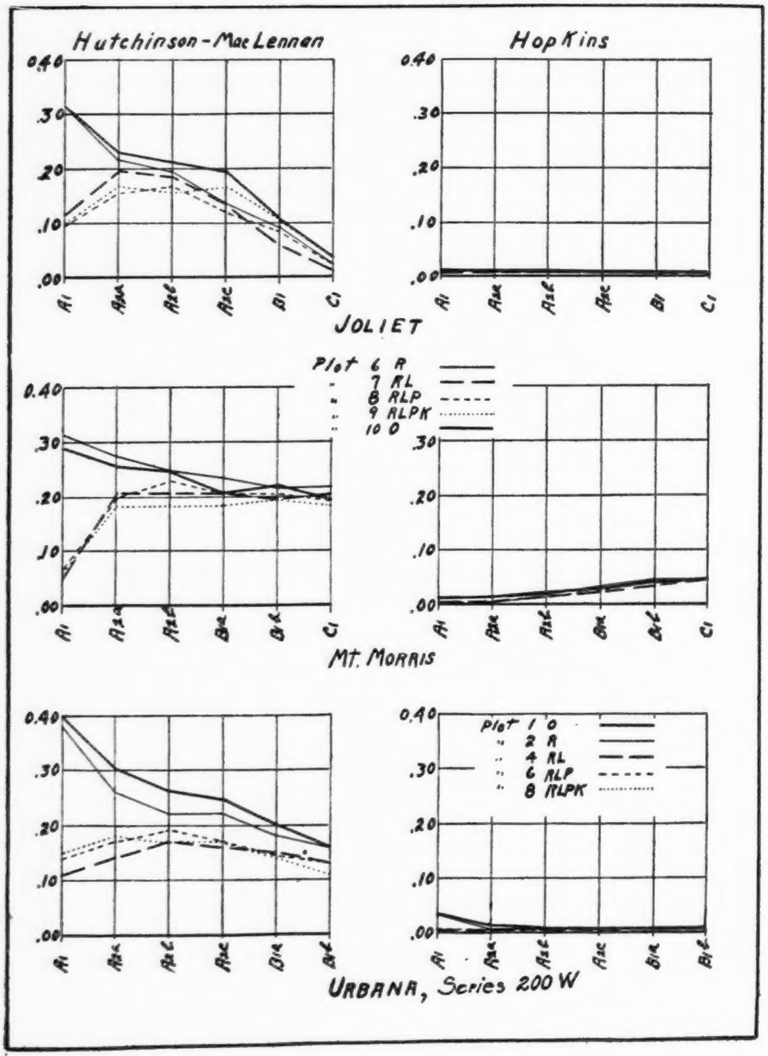

FIG. 11. EFFECT OF TREATMENTS UPON THE SOIL LIME REQUIREMENT AS MEASURED BY THE HUTCHINSON-MACLENNAN AND THE HOPKINS METHODS, IN GRAMS $CaCO_3$ PER 100 GM. SOIL

the pH to a depth greater than it has affected either total or exchangeable calcium or the soil lime-requirement is probably indicative of the greater sensitivity of the H-ion determination.

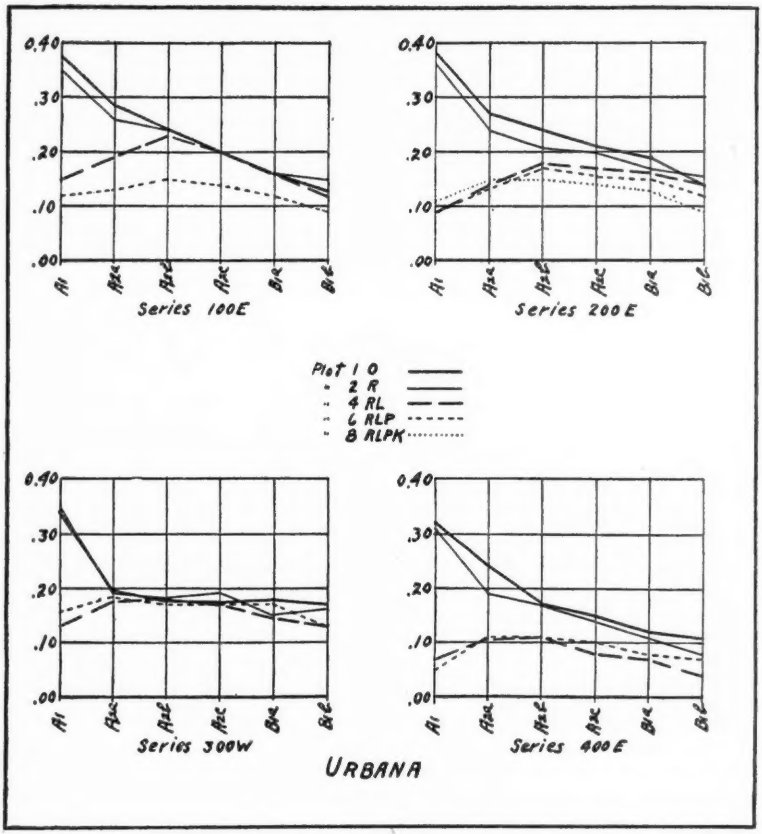

FIG. 12. EFFECT OF TREATMENTS UPON THE SOIL LIME REQUIREMENT AS MEASURED BY THE HUTCHINSON-MACLENNAN METHOD, IN GRAMS $CaCO_3$ PER 100 GM. SOIL (DATA FOR HOPKINS METHOD NOT GRAPHED)

For series 200W see figure 11

In the case of total calcium, there is some indication that the calcium in the phosphate added is revealed in the analysis of the surface horizon. The increased amounts found in phosphated plots appear to be in excess of any differences attributable to soil variation, with the exception of the soil at Urbana. In the latter case one may observe in the field a gradual change in

type from Brown Silt Loam to Black Clay Loam as one passes from plots 1 to 10, although as mapped, the Black Clay Loam is confined to the south half of plot 8, and to plots 9 and 10. This soil variation undoubtedly accounts for a considerable portion of the greater calcium content in plots 6 and 8.

The work of Page and Williams (27), on the influence of various fertilizers upon the exchangeable base content of soils from the Broadbalk field, credits manure with having the most favorable influence upon the replaceable calcium oxide content of the surface soil. Treatment consisting of superphosphate, sodium sulfate, and ammonium sulfate came second in this respect, with superphosphate and ammonium sulfate third. Other treatments had less

TABLE 5

*Total yields of all crops except corn stover and straw of small grains for the 10-year period 1917–1926, in pounds of air-dry material per acre**

FIELD	PLOT AND TREATMENT				
	6 R	7 RL	8 RLP	9 RLPK	10 O
Ewing	3,312	11,656	12,362	17,556	4,694
Toledo	5,944	13,257	14,150	16,412	6,148
Unionville	10,188	15,384	16,276	18,202	7,426
Oquawka	6,797	20,784	19,559	19,124	3,949
Joliet	18,657	19,631	24,603	26,611	16,466
Mt. Morris	21,700	31,034	32,798	34,665	19,569
Hartsburg	30,722	31,435	31,835	31,655	23,798
	1 O	2 R	4 RL	6 RLP	8 RLPK
Urbana Series 100E	32,188	34,792	43,377	48,578,	52,096
Urbana Series 200W	35,542	36,876	40,331	54,587	61,586
Urbana Series 300W	18,509	18,781	26,416	34,212	34,840
Urbana Series 400E	21,177	20,971	24,023	29,338	30,412

* The yields of the different fields are *not* comparable. One can only compare the treatments on each individual field, and, in the case of Urbana, on each individual series.

influence. It is not known to what extent natural soil variations played a part in these results.

Smith (30) treated soils representing nine different types with several salt solutions of 0.02 N and also 0.002 N concentrations. After treatment, the soils were washed thoroughly and the replaceable bases determined. The stronger of the two concentrations had a marked effect upon the absorbed base content of these soils. But the weaker solutions brought about only a very small change which, he stated, would be comparable to what might be expected from the application of soluble fertilizers in the field. Such alterations as could be observed within a period of several years would not, in his opinion, be greater than the natural variations occurring in the soil.

Relation of laboratory data to crop yields

Soil investigations, in addition to their scientific importance have added value if they bear directly or indirectly upon the crop producing capacity of the soil. It is for this reason that an effort has been made to determine the relation of crop yields to the chemical nature of the soil profile.

The combined air-dry yields of all crops in pounds per acre for the 10-year period 1917 to 1926 are given in table 5. Yields of corn stover and straw of small grains were not recorded for residue plots and hence are omitted from the table for all plots.

It is not possible to compare the total yields on one field with those of any other since no two fields were cropped exactly alike. Even if the rotations were identical it would be only by chance that the same crop was grown concurrently on two or more fields.

TABLE 6

Correlations between crop yields and chemical analysis

	HORIZON	NORTHERN FIELDS	SOUTHERN FIELDS*
Total Ca	A_1	0.266 ±0.140	0.836 ±0.049
	$A_1 + A_2a$	0.156 ±0.147	0.664 ±0.091
Exchangeable Ca	A_1	0.1006 ±0.149	0.474 ±0.127
	$A_1 + A_2a$	0.139 ±0.148	0.502 ±0.122
H. −MacL. lime requirement	A_1	−0.179 ±0.145	−0.751 ±0.071
pH	A_1	0.213 ±0.144	0.898 ±0.032

* Oquawka field included in this group.

The following weights in pounds per bushel were used in these calculations: wheat, 60; oats, 32; corn, 70; clover seed, 60; soybeans, 60; cowpeas, 60; rye, 56.

Correlations were made of crop yields with total calcium, with exchangeable calcium, with the Hutchinson-MacLennan lime-requirement results, and with the pH values, the short method recommended by Phillips (27a), being used. The data are given in table 6.

It will be seen that in the northern fields the correlations are not significant. In each case the probable error is quite large. This indicates that variations in the crop producing power of those soils are dependent upon factors other than the presence of available calcium and the absence of acidity. In other words, the soil properties measured in this study are not critical factors as related to crop production on these soils.

On the other hand, in the soils of the southern fields the correlations of crop yields with calcium and with acidity are close, particularly in the case of total calcium and of pH values. These factors are of great importance to crop

production on these soils. In field practice it is recognized that the addition of lime is usually the first and principal step to be taken in increasing the yields of the southern soils. This statement applies equally well to the Oquawka sand.

It will be observed that in the southern group the correlation of yield with exchangeable calcium is considerably lower than with total calcium. This fact can be readily explained. Liming of the soils of this group has invariably produced a larger percentage increase in exchangeable calcium than in total calcium. However, in the case of the Oquawka sand, which is included in this group, the absolute increase in exchangeable calcium has been very small in contrast to the other three soils, although the gains in crop yields have been large. Thus, the inclusion of the sand has markedly lowered the correlation coefficient. If the Oquawka sand is omitted, the correlation of crop yields with exchangeable calcium in the remaining three fields of this group is even closer than it is with total calcium, namely .887 ± .041.

Mention should be made of the correlations of yields with the total and exchangeable calcium of the first two horizons, A_1 and A_2a, combined. When this is done the correlation of yields and total calcium is lowered in both northern and southern groups. On the other hand, the correlation with *exchangeable* calcium has been raised. These facts emphasize the greater penetration of exchangeable calcium as compared to the non-exchangeable portion, and also indicate the importance of exchangeable calcium in plant growth.

No effort has been made to account quantitatively for the calcium of the lime-stone that has been applied to these soils since neither the original calcium content nor the amount removed by the crops is known.

A rather important observation which has already been alluded to (p. 154) is, that in spite of the differences noted in the soils and in the crop yields between the southern and the northern fields, the pH of the surface horizon of the soils of both groups is approximately the same, omitting Hartsburg and Oquawka. The application of limestone has decreased the exchangeable as well as the active acidity and increased the total and exchangeable calcium content. As a result the H-ion concentration has been lowered, and in the case of the southern group, the crop yield greatly augmented.

These facts indicate that the H-ion concentration of these soils may not be a primary factor in crop production. The instance of the Oquawka sand with its very low yields and yet comparatively high pH substantiates the above presumption. Odèn (25) states that, in general, the pH value at which a distinct decrease in yield occurs is probably a function of temperature and climate.

With the soils in question low base content and high exchangeable acidity due to the climate and soil age have resulted in a pH value that is accompanied by a distinct decrease in yield. In the north a somewhat differing climate and age of the soils have resulted in approximately the same pH value as above,

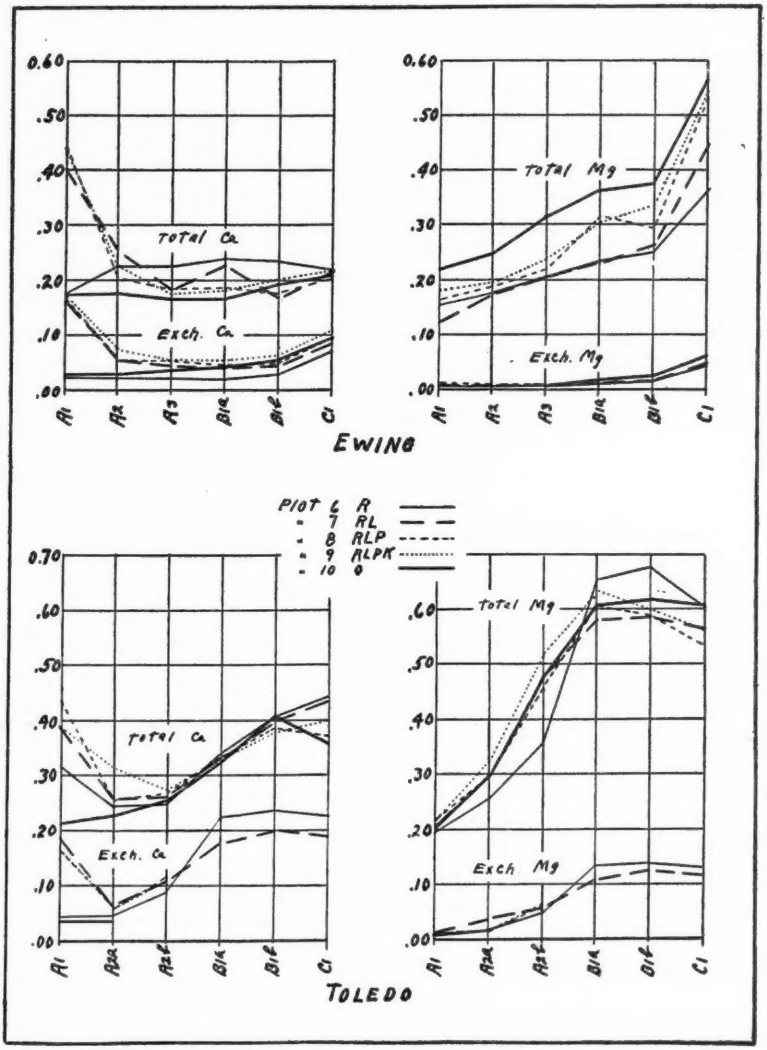

Fig. 13. Effect of Treatments upon Total and Exchangeable Calcium and Magnesium, in Grams per 100 Gm. Soil

but with a comparatively high base content, low exchangeable acidity, and high yields.

DISCUSSION

The results which have been presented in this investigation are indicative of the weathering processes that have taken place in the field. As a result of three principal factors—greater age, higher rainfall, and milder winters—the soils of the southern fields have undergone a more thorough removal of the basic constituents from the upper layers than have the soils of the north half of the state.

The Ewing and Unionville soils are characterized as having a lime requirement which increases greatly with depth, and the major portion of this acidity falls under the classification of exchangeable acidity. Hydrogen ions play an important part in the adsorption complex of these soils, particularly in the subsoil. In other words, these soils are quite unsaturated with respect to alkaline earth ions. There is a remarkable similarity in the chemical analysis of these two soils (*see* fig. 3), although their profile descriptions differ considerably. The Unionville soil is the only one used in this study which is located in an unglaciated region.

The soil from the Toledo field is in the same catagory as the Ewing and Unionville soils with respect to calcium and magnesium content, although its acidity as measured by the lime requirement methods does not increase steadily with depth as is the case with the latter soils. The type—Gray Silt Loam On Tight Clay—is characteristically variable, with the frequent occurrence of "slick spots."

The proportions of total calcium and magnesium that are in the adsorbed state are low in these southern soils, but the use of limestone on the treated plots has brought this proportion up to a figure quite comparable to that of the unlimed northern soils.

Crop yields on the untreated plots of the southern fields are usually low and are greatly enhanced by the use of limestone. This fact is well brought out in the correlation of yields with the chemical analyses. In every case the correlations are significant.

On the other hand, the soils of the northern fields are more highly buffered; they contain a greater amount of both total and exchangeable bases; and the proportion of total base that is exchangeable is much higher than is the case with the southern fields. The lime-requirement by the calcium bicarbonate method is fairly high in the surface layer, but it decreases with depth. Most of the acidity in these soils is hydrolytic rather than exchangeable.

Apparently this hydrolytic acidity is not injurious to plants. According to Gehring (15) a certain amount of exchangeable acidity in a *zeolitic-rich* soil caused no plant injury, but the same amount of acidity in a *zeolitic-poor* soil resulted in diminished plant growth. Haastert (16) likewise states that it is the exchangeable acidity which is responsible for plant injury.

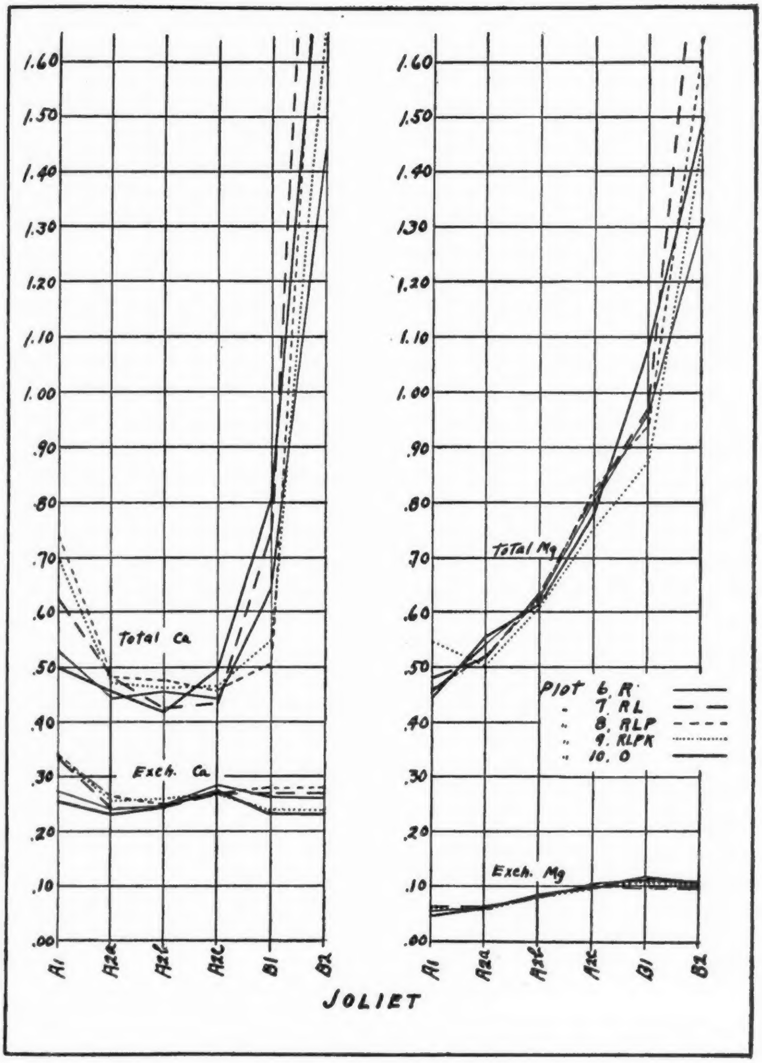

FIG. 14. EFFECT OF TREATMENTS UPON TOTAL AND EXCHANGEABLE CALCIUM AND MAGNESIUM, IN GRAMS PER 100 GM. SOIL

The Oquawka field is located in the northern part of the state, but the yields on the untreated soil are low. This sand contains almost as much calcium as does the soil on the Mt. Morris field, but the amount that is exchangeable is decidedly low in the former. The poor buffer action and the low percentage of exchangeable bases is due, of course, to the paucity of alumino-silicate complexes capable of adsorbing basic ions. The crop yields on the untreated plots of the Oquawka field are lower than one would expect from observation of the pH values and the acidity as measured by the lime-requirement methods. Therefore, it is quite probable that available calcium or calcium and magnesium are the chief limiting factors in this sand.

It is hardly necessary to refer to the Black Clay Loam at Hartsburg with its high crop yields which respond but little to soil treatment. This soil is characterized by a low acidity and a relatively high total and exchangeable calcium and magnesium content in all horizons, with the greatest amount of the set materials, excepting total magnesium, in the surface layer. The exchangeable calcium in the A_1 horizon of the unlimed plots constitutes a somewhat larger proportion of the total calcium than in the plots that have received limestone. In other words, the total calcium content—and also the carbonate calcium—was raised by the addition of limestone without increasing the exchangeable calcium to any appreciable degree. Thus, while this soil does not appear to be highly buffered (*see* table 2), this is probably because its high buffer capacity is already nearly satisfied by the high content of native soil bases.

SUMMARY

Soils from treated and untreated plots on eight Illinois experiment fields representing as many soil types were sampled by 3- to 4-inch strata. These soils were subjected to the following tests and analyses: Hydrogen-ion concentration, Comber KCNS test, buffer action, Hutchinson-MacLennan and Hopkins lime-requirement tests, total and exchangeable calcium and magnesium.

Several different solutions for the replacement of calcium and magnesium were tried, with the final adoption of ammonium acetate.

In all cases the pH values increased with depth, the increase being greatest in the Joliet soil whose subsoil is highly calcareous, and least in the soils from the Mt. Morris and Oquawka fields.

The Comber test agrees closely with the pH values.

The Oquawka sand was the most poorly buffered, whereas the Urbana, Joliet, and Mt. Morris soils possesses the greatest buffer capacity.

The Hutchinson-MacLennan lime requirement data compare favorably with electrometric titration with calcium hydroxide to a pH of approximately 7.0.

Only in the southern soils does the lime-requirement as measured by the Hutchinson-MacLennan test increase with depth. These soils are characterized by a comparatively high total acidity, of which a very large proportion

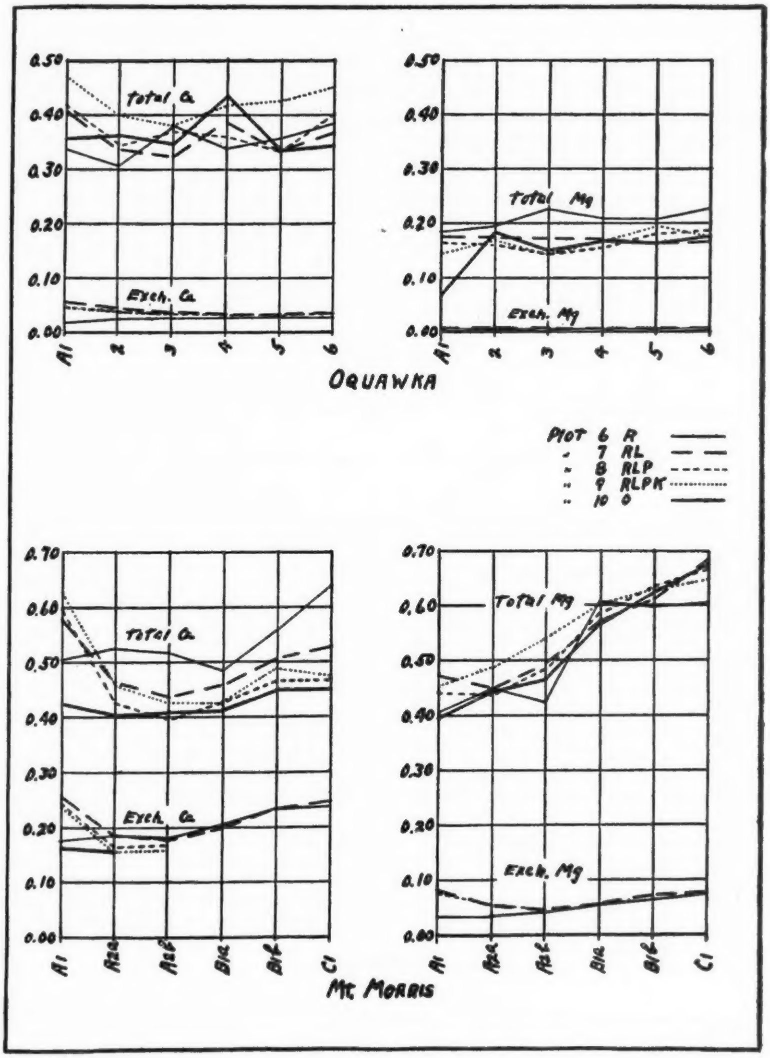

FIG. 15. EFFECT OF TREATMENTS UPON TOTAL AND EXCHANGEABLE CALCIUM AND MAGNESIUM, IN GRAMS PER 100 GM. SOIL

is the so-called exchangeable acidity, and only a small part is hydrolytic acidity.

In a very general way the pH values vary with the total calcium content, with the exception of the Black Clay Loam at Hartsburg. This soil being a

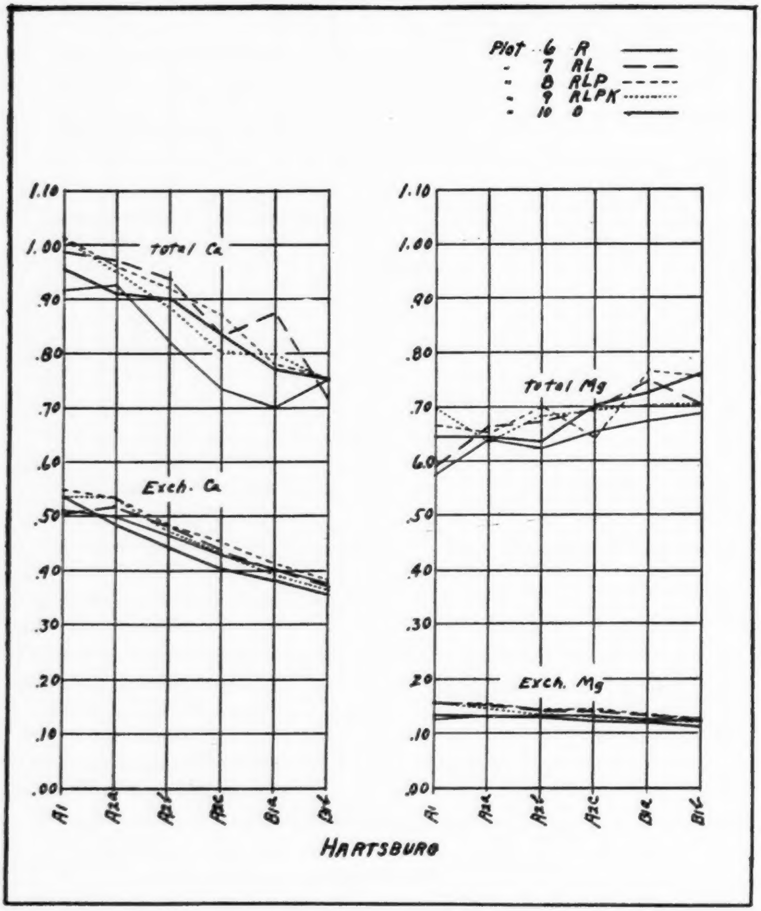

FIG. 16. EFFECT OF TREATMENTS UPON TOTAL AND EXCHANGEABLE CALCIUM AND MAGNESIUM, IN GRAMS PER 100 GM. SOIL

comparatively immature one, its calcium has not been leached out of the surface to the extent that it has in the older soils.

In most instances the total magnesium content in the surface horizon is approximately equal in amount to the calcium content in that horizon, but

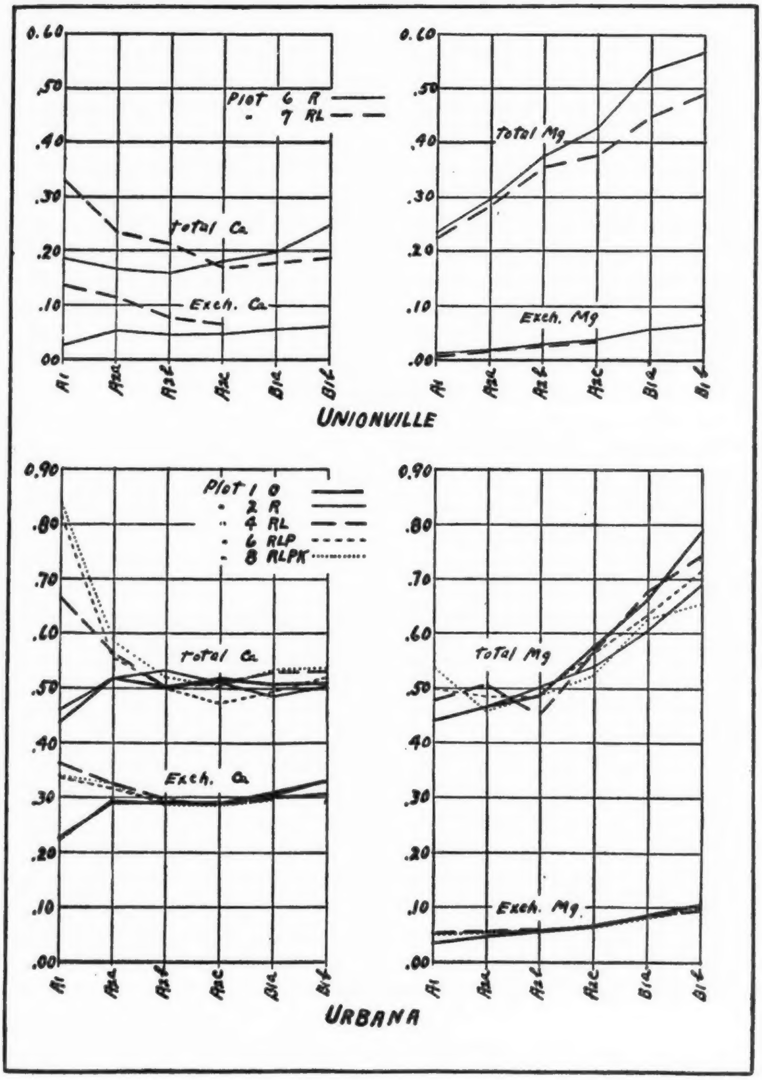

FIG. 17. EFFECT OF TREATMENTS UPON TOTAL AND EXCHANGEABLE CALCIUM AND MAGNESIUM, IN GRAMS PER 100 GM. SOIL

in the subsoils there has been a greater accumulation of magnesium than of calcium.

The curves for exchangeable calcium and magnesium follow the curves for total calcium and magnesium respectively, but the percentage of change with depth is greater with the exchangeable fraction than with the total amounts of these bases.

In the upper layers exchangesable calcium and magnesium constitute a much greater portion of the total amounts of these elements in the soils from Joliet, Mt. Morris, Urbana, and Hartsburg than is the case with the Oquawka sand and the three southern fields. Undoubtedly this is the result of the more thorough leaching that has taken place in the southern three fields due to greater age of the soils, higher rainfall, and milder winters, and in the Oquawka field due to the permeable character of the sand.

The application of limestone has reduced the H-ion concentration and the lime-requirement, and increased the total and exchangeable calcium content of the surface layer in every case.

The influence of limestone penetrated to different depths in the various soils, but only in one soil, Oquawka, was it observed below the A horizon.

This influence could be detected more readily by the pH determination than by any of the other tests.

Natural soil variations were greater than any measurable influence of treatments other than liming.

The coefficient of correlation of crop yields with total and exchangeable calcium and with acidity of the A_1 horizon is very high in the southern soils, but low in the soils from the northern fields.

The hydrogen-ion concentration of the A_1 horizon of the southern fields and three of the five northern fields was approximately identical although crop yields were considerably greater in the northern fields. The data at hand indicate that the H-ion concentration may not be a primary factor in crop production so far as its direct effect on crop growth is concerned.

In the soils of Ewing, Toledo, and Unionville, the chief chemical factors which limit crop growth are probably the presence of exchangeable acidity and the low percentage of exchangeable bases. In the Oquawka sand, an insufficient amount of available calcium appears to be the main obstacle in the way of high crop yields. With respect to the Joliet, Mt. Morris, Urbana, and Hartsburg soils, none of the factors studied can be considered as limiting.

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FIRST SUBCOMMISSION OF THE FIFTH COMMISSION, INTERNATIONAL SOCIETY OF SOIL SCIENCE MEETS AT DANZIG

HUGO CARL

Danzig

The meeting of the First Subcommission of the Fifth Commission of the International Society of Soil Science held in Danzig, May 20, was devoted to a conference about the great soil map of Europe and the problem of "Braunerde." About 50 soil scientists, representing 18 countries were present.

Dr. Stremme, president of the commission for the soil map of Europe, gave the results of his work on the map.

The maps of Russia, Poland, Czechoslovakia, Latvia, Esthonia, and Hungary are as complete as some regions of Germany. It is expected that the meeting of Leningrad in 1930 will find nine-tenths of the map finished. The soil maps of South America and the nearly complete ones of Asia that were exhibited, clearly indicated that the idea of continental soil maps is arousing interest outside Europe.

The interest of the countries was indicated in a report of Dr. Rothkegel of Berlin on the demands for the new valuation of arable land. His report stated that only through soil science is it possible to get exact and comparable methods of assessing the landed states.

Dr. Till of Wien gave details of the organization for tracing agricultural soil maps in Austria. The organization, drawn up with the help of the chambers of agriculture, is working efficiently, is cheap, and self supporting.

Prof. del Villar of Madrid gave a special report on the different soil types of Spain. The report was accompanied by a number of photos of profiles and of different types of vegetation.

The problem of "Braunerde" was treated in detail by Dr. Prassolov of Leningrad, Prof. Stebutt of Belgrade, K. Schlacht of Ludwigshafen, and Sellke of Hannover.

Schlacht presented an excellent method for the conservation of soil profiles: a piece of cardboard is covered with a special lacquer made by I. G. Farben of Ludwigshafen and is pressed for five minutes against the profile, which is thereby durably fixed.

The reports were effectively illustrated by a 3-day excursion into the districts of the Free City of Danzig.

The general discussion resulted in three resolutions being passed by the assembly: 1. A subcommission shall be appointed for the study of the coloration and methods of tracing for the soil map of Europe.

2. The types of soil around the Mediterranean Sea shall be studied by a subcommission of scientists from the bordering countries.

3. All sorts of profiles of "Braunerde" from all countries, preserved by the Schlacht or other methods shall be collected and sent to Prof. Stremme. After the study of the different types, Prof. Stremme shall give new directions for the classification and characterization of "Braunerde."

The whole meeting gave evidence of the desire on the part of all countries to perform international scientific services for the benefit of all.